A

Customer No. 20350
TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
(415) 576-0200

Attorney Docket No. 16930-0010-22

"Express Mail" Label No. EM088476313US

Date of Deposit: May 19, 1999

I hereby certify that this is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above, addressed to:

Assistant Commissioner for Patents
Washington, D.C. 20231

By: Jana Kane

ASSISTANT COMMISSIONER FOR PATENTS
BOX PATENT APPLICATION
Washington, D.C. 20231

05/19/99 U.S. PTO

Transmitted herewith for filing under 37 CFR 1.53(b) is the
[ ] patent application of
[ ] continuation patent application of
[X] divisional patent application of
[ ] continuation-in-part patent application of

05/19/99 JC518 U.S. PTO 09/315116

Inventor(s)/Applicant Identifier: **Douglas Antelman, Richard J. Gregory and Kenneth N. Wills**

For: **METHODS OF TREATING HYPERPROLIFERATIVE DISORDERS USING RETINOBLASTOMA FUSION PROTEINS**

[X] This application claims priority from each of the following Application Nos./filing dates:
08/801,092 filed February 14, 1997; 08/751,517 filed November 15, 1996,
the disclosure(s) of which is (are) incorporated by reference.

[X] Please amend this application by adding the following before the first sentence: "This application is a division of and claims the benefit of U.S. Application No. 08/801,092 filed February 14, 1997, which is a continuation of and claims the benefit of U.S. Application No. 08/751,517 filed November 15, 1996, the disclosures of which are incorporated by reference."

Enclosed are:

[X] 31 page(s) of specification
[X] 4 page(s) of claims
[X] 1 page of Abstract
[X] 51 sheet(s) of [ ] formal [X] informal drawing(s).
[X] An assignment of the invention to Canji, Inc. was recorded in the prior application
[X] A [X] signed [ ] unsigned Declaration & Power of Attorney **(copy from prior application)**
[X] A Preliminary Amendment.
[X] Sequence Listing pages 32-62

Claims after Entry of any Amendments, Less any Canceled Claims

	(Col. 1)		(Col. 2)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
FOR:	NO. FILED		NO. EXTRA	RATE	FEE		RATE	FEE
BASIC FEE					$380.00	OR		$760.00
TOTAL CLAIMS	22	- 20	= *2	x $9.00 =		OR	x $18.00 =	$ 36.00
INDEP. CLAIMS	1	- 3	= *0	x $39.00 =		OR	x $78.00 =	$0.00
[ ] MULTIPLE DEPENDENT CLAIM PRESENTED				+ $130.00 =		OR	+ $260.00 =	
				TOTAL		OR	TOTAL	$760.00

* If the difference in Col. 1 is less than 0, enter "0" in Col. 2.

Please charge Deposit Account No. 20-1430 as follows:

[X] Filing fee $ $796.00
[X] Any additional fees associated with this paper or during the pendency of this application.
[ ] The issue fee set in 37 CFR 1.18 at or before mailing of the Notice of Allowance, pursuant to 37 CFR 1.311(b)

[ ] A check for $_____ is enclosed.
2 extra copies of this sheet are enclosed.

Respectfully submitted,
TOWNSEND and TOWNSEND and CREW LLP

Timothy L. Smith
Reg No.: 35,367
Attorneys for Applicant

Telephone:
(415) 576-0200

Facsimile:
(415) 576-0300

"Express Mail" Label No. EM088476313US
Date of Deposit: May 19, 1999

I hereby certify that this is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above, addressed to:

Assistant Commissioner for Patents
Washington, D.C. 20231
By: _____

PATENT
Attorney Docket No. 16930-001022US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Douglas Antelman *et al.*

Serial No.: not yet assigned

Filed: May 19, 1999

For: METHODS OF TREATING HYPERPROLIFERATIVE DISORDERS USING RETINOBLASTOMA FUSION PROTEINS

Examiner: Lin Sun-Hoffman, Ph.D.
(parent)

Art Unit: 1642 (parent)

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Applicant respectfully requests entry of the following amendments prior to examination of the application.

In the Title:

Please delete the title and insert therefor: --METHODS OF TREATING HYPERPROLIFERATIVE DISORDERS USING RETINOBLASTOMA FUSION PROTEINS--.

In the Specification:

At page 2, line 5, please delete "518-521" and insert --518-522--.

At page 3, line 17, after "Figure 1A" insert --(SEQ ID NO:1)--.

At page 3, line 19, after "Figure 1B" insert --(SEQ ID NO:2)--.

At page 3, line 21, after "Figure 2A" insert --(SEQ ID NO:3)--.

At page 3, line 23, after "Figure 2B" insert --(SEQ ID NO:4)--.

At page 3, line 26, after "Figure 4" insert --(SEQ ID NOS:5-18)--.

At page 3, line 32, after "Figure 8" insert --(SEQ ID NOS:33-46)--.

At page 3, line 19, after "Figure 1A" insert --(SEQ ID NO:2)--.

At page 10, line 9, please delete "Hartzoglou" and insert --Hatzoglou--.

At page 11, at line 18, please delete "1098" and insert --1089--. At line 19, please delete "Casper" and insert --Kasper--. At line 24, please delete "Tanura" and insert --Tamura--.

At page 19, line 12, please delete "11" and insert --111--. At line 20, please delete "Willart" and insert --Willard--. At line 21, please delete "1995" and insert --1994--.

At page 27, line 6, please delete "Thimmappaaya" and insert --Thimmappaya--.

After page 31, please insert the enclosed paper copy of the sequence listing, which consists of pages 32-62. Please renumber the following pages accordingly.

In the Claims:

Please cancel claims 1-15.

Please amend claims 16, 17, 22-26 and 32-33 as follows:

16. (Once amended) A method for treatment of a hyperproliferative disorder in a patient, the method comprising administering to a patient a therapeutically effective dose of a fusion polypeptide that comprises [comprising a fusion of] a DNA binding domain of a transcription factor and a functional growth suppression domain of a [, the transcription factor comprising a DNA binding domain, and a] retinoblastoma (RB) polypeptide [, the RB polypeptide comprising a growth suppression domain].

17. (Once amended) The method of claim 16, wherein the fusion polypeptide [protein] is encoded by a nucleic acid delivered to the patient.

18. (Reiterated) The method of claim 16, wherein the transcription factor is E2F.

19. (Reiterated) The method of claim 18, wherein the cyclin A binding domain of the E2F is deleted or nonfunctional.

20. (Reiterated) The method of claim 16, wherein the RB is RB56.

21. (Reiterated) The method of claim 16, wherein the RB is wild type RB56.

22. (Once amended) The method of claim 16, wherein the functional growth suppression domain of the RB polypeptide comprises from about amino acid residue 379 to about amino acid residue 928 (SEQ ID NO:4).

23. (Once amended) The method of claim 16, wherein the functional growth suppression domain of the RB polypeptide comprises at least one substitution of amino acid residues selected from the group consisting of 2, 608, 612, 788, 807, and 811.

24. (Once amended) The method of claim 18, wherein the E2F polypeptide comprises about amino acid residues 95 to about 286 (SEQ ID NO:1).

25. (Once amended) The method of claim 18, wherein the E2F polypeptide comprises about amino acid residues 95 to about 194 (SEQ ID NO:1).

26. (Once amended) The method of claim 16, wherein the fusion polypeptide comprises EF2 amino acid residues from about 95 to about 194 (SEQ ID NO:1) operatively linked to RB amino acid residues from about 379 to about 928 (SEQ ID NO:4).

27. (Reiterated) The method of claim 18, wherein the E2F-RB fusion polypeptide is expressed under the control of a tissue-specific promoter.

28. (Reiterated) The method of claim 27, wherein the tissue specific promoter is a smooth muscle actin promoter.

29. (Reiterated) The method of claim 16, wherein the hyperproliferative disorder is cancer.

30. (Reiterated) The method of claim 29, wherein the cancer is bladder cancer.

31. (Reiterated) The method of claim 29, wherein the hyperproliferative disorder is restenosis.

32. (Once amended) The method of claim 31, wherein the [E2F-RB] fusion polypeptide is administered after angioplasty.

33. (Once amended) The method of claim 32, wherein the [E2F-RB] fusion polypeptide is administered as a coating on an angioplasty device.

34. (Reiterated) The method of claim 17, wherein the nucleic acid is administered after angioplasty.

35. (Reiterated) The method of claim 17, wherein the nucleic acid is administered as a coating on an angioplasty device.

36. (Reiterated) The method of claim 17, wherein the nucleic acid is inserted in an adenovirus vector.

Please add the following new claim 37.

37. The method of claim 16, wherein the fusion polypeptide lacks a functional cyclin A-kinase binding domain of the transcription factor.

REMARKS

Status of the Application

Claims 16-37 are pending with entry of this amendment, with claims 16-36 previously in the application and entry of new claim 37 respectfully requested.

The Amendments

The amendments to page 3 of the specification provide the sequence ID numbers for the various amino acid and nucleotide sequences. The remaining amendments to the specification correct various errors in the citations of the listed references.

The Sequence Listing

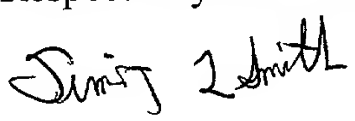
The paper copy of the Sequence Listing in this application is identical to the computer readable copy of the Sequence Listing filed in Application No. 08/801,092, filed February 14, 1997. In accordance with 37 CFR § 1.821(e), please use the only computer readable form filed in that application (filed May 12, 1997) as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the instant application. A paper copy of the Sequence Listing is enclosed herewith for incorporation into the specification. Applicants attest that the information contained in the Sequence Listing introduces no new matter and that the computer-readable form submitted herewith is the same as the paper copy of the Sequence Listing.

CONCLUSION

In view of the foregoing, Applicants believe that all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned attorney at (415) 576-0200.

Respectfully submitted,



Timothy L. Smith, Ph.D.
Reg. No. 35,367

TOWNSEND and TOWNSEND and CREW, LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
(415) 576-0200
(415) 576-0300 (facsimile)

::odma\pcdocs\sf\221927\1

Attorney Docket No. 16930-001020
Client Ref. 3015

PATENT APPLICATION

FOR

TISSUE SPECIFIC EXPRESSION OF RETINOBLASTOMA PROTEIN

Inventors:

Douglas Antelman, a citizen of the United States of America, residing at 1716 Swallowtail Road, Encinitas, California 92024;

Richard J. Gregory, a citizen of United States of America, residing at 2 Wintergreen Lane, Westford, Massachusetts 01886;

Kenneth N. Wills, a citizen of the United States of America, residing at 821 Bluffcrest Lane, Encinitas, California 92024.

Assignee:

Canji, Inc.
3030 Science Park Road
San Diego, California
A Delaware Corporation.

Entity: Large Entity

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
(415) 326-2400

PATENT
Attorney Docket No. 16930-001020
Client Ref. 3015

TISSUE SPECIFIC EXPRESSION OF RETINOBLASTOMA PROTEIN

BACKGROUND OF THE INVENTION

Both the retinoblastoma gene (RB) and transcription factor E2F play a critical role in cell growth control (for a review, see Adams, P. & Kaelin, W. Seminars in Cancer Biology 6:99-108 (1995)). The RB locus is frequently inactivated in a variety of human tumor cells. Reintroduction of a wild-type RB gene (e.g., Bookstein et al. Science 247:712-715 (1990)) or RB protein (pRB) (e.g., Antelman et al. Oncogene 10:697-704(1995)) into RBneg/RBmut cells can suppress growth in culture and tumorigenicity *in vivo*.

While E2F serves to activate transcription of S-phase genes, its activity is kept in check by RB. RB arrests cells by blocking exit from G into S-phase (for example, Dowdy et al. Cell 73:499-511 (1993)) but the precise pathway of the arrest remains unclear.

Although E2F forms complexes with RB, complex formation is more efficient if an E2F-related protein, DP-1, is present. E2F-1 and DP-1 form stable heterodimers which bind to DNA (for example, Qin et al. Genes and Dev. 6-:953-964 (1992)). DP-1-E2F complexes serve to cooperatively activate transcription of E2F-dependent genes. Such transcription can be repressed by pRB in the same manner as E2F-1 or DP-1 activated transcription.

Transcriptional repression of genes by RB in some instances can be achieved by tethering pRB to a promoter. For example, GAL4-pRB fusions bind to GAL4 DNA binding domains and repress transcription from p53, Sp-1 or AP-1 elements (Adnane, et al. J. Biol. Chem. 270:8837-8843 (1995); Weintraub, et al.

Nature 358:259-261 (1995)). Sellers, et al. (Proc. Natl. Acad. Sci. 92:11544-11548 (1995)) disclosed fusions of amino acid residues 1-368 of E2F with amino acids 379-792 or 379-928 of RB.

Chang, et al. (Science 267:518-521 (1995)) disclosed the use of a replication-defective adenovirus-RB construct in the reduction of neointima formation in two animal models of restenosis, a hyperproliferative disorders.

SUMMARY OF THE INVENTION

The instant invention provides the surprising result that a fusion of an E2F polypeptide with an RB polypeptide is more efficient in repressing transcription of the E2F promoter than RB alone, and that such fusions can cause cell cycle arrest in a variety of cell types. Such fusions can thus address the urgent need for therapy of hyperproliferative disorders, including cancer.

One aspect of the invention is a polypeptide comprising a fusion of a transcription factor, the transcription factor comprising a DNA binding domain, and a retinoblastoma (RB) polypeptide, the RB polypeptide comprising a growth suppression domain. Another aspect of the invention is DNA encoding such a fusion polypeptide. The DNA can be inserted in an adenovirus vector.

In some embodiments of the invention, the transcription factor is E2F. The cyclin A binding domain of the E2F can be deleted or nonfunctional. The E2F can comprise amino acid residues about 95 to about 194 or about 95 to about 286 in some embodiments.

The retinoblastoma polypeptide can be wild-type RB, RB56, or a variant or fragment thereof. In some embodiments, the retinoblastoma polypeptide comprises amino acid residues of about 379 to about 928. Preferred amino acid substitutions of the RB polypeptide include residues 2, 608, 788, 807, and 811.

Another aspect of the invention is an expression vector comprising DNA encoding a polypeptide, the polypeptide comprising a fusion of a transcription factor, the

transcription factor comprising a DNA binding domain, and a retinoblastoma (RB) polypeptide, the RB polypeptide comprising a growth suppression domain. In some embodiments a tissue-specific promoter is operatively linked to DNA encoding the fusion polypeptide. The tissue-specific promoter can be a smooth muscle alpha actin promoter.

Another aspect of the invention is a method for treatment of hyperproliferative disorders comprising administering to a patient a therapeutically effective dose of an E2F-RB fusion polypeptide. The hyperproliferative disorder can be cancer. In some embodiments the hyperproliferative disorder is restenosis. The fusion polypeptide and nucleic acid encoding the fusion polypeptide can be used to coat devices used for angioplasty.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A depicts the predicted amino acid sequence of E2F.

Figure 1B depicts the nucleotide sequence of transcription factor E2F.

Figure 2A depicts the nucleotide sequence of pRB as disclosed by Lee, et al. (Nature 329:642-645 (1987).

Figure 2B depicts the predicted amino acid sequence of pRB.

Figure 3 is a diagrammatic representation of pCTM.

Figure 4 depicts the nucleotide sequence of plasmid pCTM.

Figure 5 is a diagrammatic representation of pCTMI.

Figure 6 depicts the nucleotide sequence of pCTMI.

Figure 7 is a diagrammatic representation of plasmid pCTMIE.

Figure 8 depicts the nucleotide sequence of pCTMIE.

Figure 9 is a diagram depicting E2F-RB fusion constructs used in the examples. All E2F constructs commenced at amino acid 95 and lacked part of the cyclin A binding domain. E2F-437 contained the DNA binding domain (black), heterodimerization domain (white), and the transactivation domain (stippled). E2F-194 contained solely the DNA binding

domain. E2F-286 contained the DNA binding domain and the DP-1 heterodimerization domain. To generate E2F-194-RB56-5s and E2F-286-RB56-5s, the E2F constructs were fused in-frame to codon 379 of RB. C706F is an inactivating point mutation.

Figure 10 is a diagram depicting transcriptional repression by E2F-RB fusion constructs.

Figure 11 (A-D) depicts expression of E2F-RB fusion proteins in mammalian cell lines. Extracts were prepared from cells used in E2-CAT reporter assays or in FACS assays and analyzed with an anti-RB monoclonal antibody. In panel A, the results are shown from C33A cells transfected with (3) RB56-H209, (4) RB56 wild-type, (5) RB56-5s, (6) E2F286-5s, (7) E2F194-5s, (8) E2F194, (9) E2F286, (10) E2F437. Lane (1) is an RB56 protein standard. Lane (2) is a mock transfection. In panel B, results are shown for transfection of Saos-2 cells with (1) RB56, (2,3) E2F194-5s, and (4,5) E2F286-5s. In panel C, results are shown for transfection of 5637 cells with (2,3) RB56 wild-type, (4,5) RB56-5s; (6,7) E2F194-5s; (7,8) E2F286-5S. Lane (1) is an RB56 protein standard. In panel D, results are shown for NIH-3T3 transfected (3) RB56, (4) E2F286-5s, (5) E2F194-5s. Lane (1) is an RB56 standard; lane (2) is an RB110 standard.

Figure 12 depicts histogram analyses of flow cytometry of RB-expressing NIH-3T3 cells.

Figure 13, panel A, depicts a comparison of the effects of a CMV-driven recombinant adenovirus (ACN56) with two isolates of a human smooth muscle alpha actin-driven E2F-p56 fusion construct consisting of amino acids 95 through 286 of E2F linked directly and in-frame to p56 (amino acids 379-928 of RB cDNA), vs. a control virus (ACN) in a ^{3}H-thymidine uptake assay in the rat smooth muscle cell line A7R5. Panel (B) depicts the effects of the same constructs in the rat smooth muscle cell line A10.

Figure 14 depicts a comparison of the effects of the viruses described in Fig. 13 in non-muscle cells. Panel (A) depicts results in the breast carcinoma cell line MDA MB468. Panel (B) depicts results in the non-small cell lung cell carcinoma line H358.

Figure 15, top panel, depicts the relative infectivity by adenovirus of different cell lines as judged by the level of β-galactosidase (β-gal) staining following infection with equal amounts of a recombinant adenovirus expressing β-gal driven by a CMV promoter. H358 is non-small lung cell carcinoma cell line; MB468 is a breast carcinoma cell line; A7R5 and A10 are smooth muscle cell lines. The lower portion of the figure depicts the relative levels of p56 protein expressed in the same cells when infected with the recombinant adenovirus ACN56, in which the p56 cDNA is driven by the non-tissue specific CMV promoter.

Figure 16 depicts relative protein levels in cells infected with the smooth muscle alpha actin promoter-driven E2F-p56 fusion construct (ASN286-56). UN denoted uninfected; 50, 100, 250, and 500 refer to multiplicities of infection (MOI).

Figure 17 is a bar graph depicting the ratio of intima to media area (as a measurement of the inhibition of neointima formation) from cross-sections (n=9) of rat carotid arteries which were injured and treated with recombinant adenoviruses expressing either β-gal, RB (ACNRB) or p56 (ACN56), all under the control of the CMV promoter.

Figure 18 is a series of three photographs depicting restenosis in a rat angioplasty model. The panel on the left depicts data from a normal animal; the central panel depicts data from an animal injured and then treated with a β-gal expressing recombinant virus; the panel on the right depicts data from an animal injured and then treated with a recombinant adenovirus expressing p56 (ACN56).

Figure 19 depicts tissue-specificity of the smooth muscle alpha actin promoter, as demonstrated by its selective ability to express the β-gal transgene in muscle cells but not non-muscle cells. The panels on the left compare β-gal expression in the breast cell carcinoma line MB468 infected with either an MOI=1 with a CMV-driven β-gal (ACNBGAL) vs an MOI= 100 with the smooth muscle promoter construct (ASNBGAL). The panels on the right show β-gal expression of the rat smooth muscle cell line A7R5 infected with either an MOI=1 of

ACNBGAL or an MOI=50 of ASNBGAL. Expression from ASNBGAL is seen in the muscle cell line, but is absent in the non-muscle cell line, despite the higher degree of infectivity of the cells.

Figure 20 depicts the ability of recombinant adenovirus expressing RB to transduce rat carotid arteries. recombinant adenovirus-treated arteries (1X 10^9 pfu) were harvested two days following balloon injury and infection. Cross sections were fixed and an RB specific antibody was used to detect the presence of RB protein in the tissue. The control virus used was ACN. RB protein staining was evident in the ACNRB treated sample, especially at higher magnifications.

Figure 21 depicts a comparison of the effects of a CMV-driven p56 recombinant adenovirus (ACN56E4) vs a human smooth muscle alpha-actin promoter-driven E2F-p56 fusion construct (ASN286-56) vs control adenoviral constructs containing either the CMV or smooth muscle alpha-actin promoters without a downstream transgene (ACNE3 or ASBE3-2 isolates shown, respectively). Assays were ^{3}H-thymidine uptake either in a smooth muscle cell line (A7R5) or a non-muscle cell line (MDA-MB468, breast carcinoma). Results demonstrated muscle tissue specificity using the smooth muscle alpha-actin promoter and specific inhibition by both the p56 and E2F-p56 transgenes relative to their respective controls.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The instant invention provides RB fusion constructs including fusion polypeptides and vectors encoding them, and methods for the use of such constructs in the treatment of hyperproliferative diseases. In some preferred embodiments of the invention, an RB polypeptide is fused to an E2F polypeptide. Any E2F species can be used, typically E2F-1, -2, -3, -3, or -5 (see, e.g., Wu et al. Mol Cell. Biol. 15:2536-2546 (1995); Ivey-Hoyle et al. Mol. Cell. Biol. 13:7802 (1993); Vairo et al. Genes and Dev. 9:869 (1995); Beijersbergen et al. Genes and Dev. 8:2680 (1994)); Ginsberg

et al. Genes and Dev. 8:2665 (1994); Buck et al. Oncogene 11:31 (1995)), more typically E2F-1. Typically, the EF2 polypeptide comprises at least the DNA binding domain of E2F, and may optionally include the cyclin A binding domain, the heterodimerization domain, and/or the transactivation domain. Preferably, the cyclin A binding domain is not functional. The nucleotide and amino acid sequence of E2F referred to herein are those of Genbank HUME2F, shown in Figure 1A and 1B. Nucleic acid, preferably DNA, encoding such an EF2 polypeptide is fused in reading frame to an RB polypeptide. The RB polypeptide can be any RB polypeptide, including conservative amino acid variants, allelic variants, amino acid substitution, deletion, or insertion mutants, or fragments thereof. Preferably, the growth suppression domain, i.e., amino acids residues 379-928, of the RB polypeptide is functional (Hiebert, et al. MCB **13:3384-3391** (1993); Qin, et al. Genes and Dev. **6:953-964** (1992)). In some embodiments, wild-type pRB110 is used. More preferably, a truncated version of RB, RB56, is used. RB56 comprises amino acid residues 379-928 of pRB110 (Hiebert, et al. MCB **13:3384-3391** (1993); Qin, et al. Genes and Dev. **6:953-964** (1992)). In some embodiments, amino acid variants of RB at positions 2, 608, 612, 788, 807, or 811, are used singly or in combination. The variant RB56-5s comprises wild-type RB56 having alanine substitutions at 608, 612, 788, 807, and 811. Numbering of RB amino acids and nucleotides is according to the RB sequence disclosed by Lee, et al. (Nature **329:642-645** (1987)), hereby incorporated by reference in its entirety for all purposes. (Figure 2).

Nucleic acids encoding the polypeptides of the invention can be DNA or RNA. The phrase "nucleic acid sequence encoding" refers to a nucleic acid which directs the expression of a specific protein or peptide. The nucleic acid sequences include both the DNA strand sequence that is transcribed into RNA and the RNA sequence that is translated into protein. The nucleic acid sequences include both the full length nucleic acid sequences as well as non-full length

sequences derived from the full length protein. It is further understood that the sequence includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell.

The term "vector" as used herein refers to viral expression systems, autonomous self-replicating circular DNA (plasmids), and includes both expression and nonexpression plasmids. Where a recombinant microorganism or cell culture is described as hosting an "expression vector," this includes both extrachromosomal circular DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or is incorporated within the host's genome. A vector contains multiple genetic elements positionally and sequentially oriented, i.e., operatively linked with other necessary elements such that nucleic acid in the vector encoding the constructs of the invention can be transcribed, and when necessary, translated in transfected cells.

The term "gene" as used herein is intended to refer to a nucleic acid sequence which encodes a polypeptide. This definition includes various sequence polymorphisms, mutations, and/or sequence variants wherein such alterations do not affect the function of the gene product. The term "gene" is intended to include not only coding sequences but also regulatory regions such as promoters, enhancers, and termination regions. The term further includes all introns and other DNA sequences spliced from the mRNA transcript, along with variants resulting from alternative splice sites.

The term "plasmid" refers to an autonomous circular DNA molecule capable of replication in a cell, and includes both the expression and nonexpression types. Where a recombinant microorganism or cell culture is described as hosting an "expression plasmid", this includes both extrachromosomal circular DNA molecules and DNA that has been incorporated into the host chromosome(s). Where a plasmid is being maintained by a host cell, the plasmid is either being

stably replicated by the cells during mitosis as an autonomous structure or is incorporated within the host's genome.

The phrase "recombinant protein" or "recombinantly produced protein" refers to a peptide or protein produced using non-native cells that do not have an endogenous copy of DNA able to express the protein. The cells produce the protein because they have been genetically altered by the introduction of the appropriate nucleic acid sequence. The recombinant protein will not be found in association with proteins and other subcellular components normally associated with the cells producing the protein. The terms "protein" and "polypeptide" are used interchangeably herein.

In general, a construct of the invention is provided in an expression vector comprising the following elements linked sequentially at appropriate distances for functional expression: a tissue-specific promoter, an initiation site for transcription, a 3' untranslated region, a 5' mRNA leader sequence, a nucleic acid sequence encoding a polypeptide of the invention, and a polyadenylation signal. Such linkage is termed "operatively linked." Enhancer sequences and other sequences aiding expression and/or secretion can also be included in the expression vector. Additional genes, such as those encoding drug resistance, can be included to allow selection or screening for the presence of the recombinant vector. Such additional genes can include, for example, genes encoding neomycin resistance, multi-drug resistance, thymidine kinase, beta-galactosidase, dihydrofolate reductase (DHFR), and chloramphenicol acetyl transferase.

In the instant invention, tissue-specific expression of the RB constructs of the invention is preferably accomplished by the use of a promoter preferentially used by a tissue of interest. Examples of tissue-specific promoters include the promoter for creatine kinase, which has been used to direct the expression of dystrophin cDNA expression in muscle and cardiac tissue (Cox, et al. Nature **364:725-729** (1993)) and immunoglobulin heavy or light chain promoters for the expression of suicide genes in B cells (Maxwell, et al. Cancer Res. **51:4299-4304** (1991)). An endothelial cell-

specific regulatory region has also been characterized (Jahroudi, et al. Mol. Cell. Biol. 14:999-1008 (1994)).

Amphotrophic retroviral vectors have been constructed carrying a herpes simplex virus thymidine kinase gene under the control of either the albumin or alpha-fetoprotein promoters (Huber, et al. Proc. Natl. Acad. Sci. U.S.A. 88:8039-8043 (1991)) to target cells of liver lineage and hepatoma cells, respectively. Such tissue specific promoters can be used in retroviral vectors (Hartzoglou, et al. J. Biol. Chem. 265:17285-17293 (1990)) and adenovirus vectors (Friedman, et al. Mol. Cell. Biol. 6:3791-3797 (1986); Wills et al. Cancer Gene Therapy 3:191-197 (1995)) and still retain their tissue specificity.

In the instant invention, a preferred promoter for tissue-specific expression of exogenous genes is the human smooth muscle alpha-actin promoter. Reddy, et al. (J. Cell Biology 265:1683-1687 (1990)) disclosed the isolation and nucleotide sequence of this promoter, while Nakano, et al. (Gene 99:285-289 (1991)) disclosed transcriptional regulatory elements in the 5' upstream and the first intron regions of the human smooth muscle (aortic type) alpha-actin gene.

Petropoulos, et al. (J. Virol. 66:3391-3397 (1992)) disclosed a comparison of expression of bacterial chloramphenicol transferase (CAT) operatively linked to either the chicken skeletal muscle alpha actin promoter or the cytoplasmic beta-actin promoter. These constructs were provided in a retroviral vector and used to infect chicken eggs.

Exemplary tissue-specific expression elements for the liver include but are not limited to HMG-CoA reductase promoter (Luskey, Mol. Cell. Biol. 7(5):1881-1893 (1987)); sterol regulatory element 1 (SRE-1; Smith et al. J. Biol. Chem. 265(4):2306-2310 (1990); phosphoenol pyruvate carboxy kinase (PEPCK) promoter (Eisenberger et al. Mol. Cell Biol. 12(3):1396-1403 (1992)); human C-reactive protein (CRP) promoter (Li et al. J. Biol. Chem. 265(7):4136-4142 (1990));

human glucokinase promoter (Tanizawa et al. Mol. Endocrinology **6(7):1070-81** (1992); cholesterol 7-alpha hydroylase (CYP-7) promoter (Lee et al. J. Biol. Chem. **269(20):14681-9** (1994)); beta-galactosidase alpha-2,6 sialyltransferase promoter (Svensson et al. J. Biol. Chem. **265(34):20863-8** (1990); insulin-like growth factor binding protein (IGFBP-1) promoter (Babajko et al. Biochem Biophys. Res. Comm. **196 (1):480-6** (1993)); aldolase B promoter (Bingle et al. Biochem J. **294(Pt2):473-9** (1993)); human transferrin promoter (Mendelzon et al. Nucl. Acids Res. **18(19):5717-21** (1990); collagen type I promoter (Houglum et al. J. Clin. Invest. **94(2):808-14** (1994)).

Exemplary tissue-specific expression elements for the prostate include but are not limited to the prostatic acid phosphatase (PAP) promoter (Banas et al. Biochim. Biophys. Acta. **1217(2):188-94** (1994); prostatic secretory protein of 94 (PSP 94) promoter (Nolet et al. Biochim. Biophys. ACTA **1098(2):247-9** (1991)); prostate specific antigen complex promoter (Casper et al. J. Steroid Biochem. Mol. Biol. **47 (1-6):127-35** (1993)); human glandular kallikrein gene promoter (hgt-1) (Lilja et al. World J. Urology **11(4):188-91** (1993).

Exemplary tissue-specific expression elements for gastric tissue include but are not limited to the human H^+/K^+-ATPase alpha subunit promoter (Tanura et al. FEBS Letters **298:(2-3):137-41** (1992)).

Exemplary tissue-specific expression elements for the pancreas include but are not limited to pancreatitis associated protein promoter (PAP) (Dusetti et al. J. Biol. Chem. **268(19):14470-5** (1993)); elastase 1 transcriptional enhancer (Kruse et al. Genes and Development **7(5):774-86** (1993)); pancreas specific amylase and elastase enhancer promoter (Wu et al. Mol. Cell. Biol. **11(9):4423-30** (1991); Keller et al. Genes & Dev. **4(8):1316-21** (1990)); pancreatic cholesterol esterase gene promoter (Fontaine et al. Biochemistry **30(28):7008-14** (1991)).

Exemplary tissue-specific expression elements for the endometrium include but are not limited to the uteroglobin promoter (Helftenbein et al. Annal. NY Acad. Sci. 622:69-79 (1991)).

Exemplary tissue-specific expression elements for adrenal cells include but are not limited to cholesterol side-chain cleavage (SCC) promoter (Rice et al. J. Biol. Chem. 265:11713-20 (1990).

Exemplary tissue-specific expression elements for the general nervous system include but are not limited to gamma-gamma enolase (neuron-specific enolase, NSE) promoter (Forss-Petter et al. Neuron 5(2):187-97 (1990)).

Exemplary tissue-specific expression elements for the brain include but are not limited to the neurofilament heavy chain (NF-H) promoter (Schwartz et al. J. Biol. Chem. 269(18):13444-50 (1994)).

Exemplary tissue-specific expression elements for lymphocytes include but are not limited to the human CGL-1/granzyme B promoter (Hanson et al. J. Biol. Chem. 266 (36):24433-8 (1991)); the terminal deoxy transferase (TdT), lambda 5, VpreB, and lck (lymphocyte specific tyrosine protein kinase p561ck) promoter (Lo et al. Mol. Cell. Biol. 11(10):5229-43 (1991)); the humans CD2 promoter and its 3'transcriptional enhancer (Lake et al. EMBO J. 9(10):3129-36 (1990)), and the human NK and T cell specific activation (NKG5) promoter (Houchins et al. Immunogenetics 37(2):102-7 (1993)).

Exemplary tissue-specific expression elements for the colon include but are not limited to pp60c-src tyrosine kinase promoter (Talamonti et al. J. Clin. Invest 91(1):53-60 (1993)); organ-specific neoantigens (OSNs), mw 40kDa (p40) promoter (Ilantzis et al. Microbiol. Immunol. 37(2):119-28 (1993)); colon specific antigen-P promoter (Sharkey et al. Cancer 73(3 supp.) 864-77 (1994)).

Exemplary tissue-specific expression elements for breast cells include but are not limited to the human alpha-

lactalbumin promoter (Thean et al. British J. Cancer, 61(5):773-5 (1990)).

Other elements aiding specificity of expression in a tissue of interest can include secretion leader sequences, enhancers, nuclear localization signals, endosmolytic peptides, etc. Preferably, these elements are derived from the tissue of interest to aid specificity.

Techniques for nucleic acid manipulation of the nucleic acid sequences of the invention such as subcloning nucleic acid sequences encoding polypeptides into expression vectors, labelling probes, DNA hybridization, and the like are described generally in Sambrook *et al.*, Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, (1989), which is incorporated herein by reference. This manual is hereinafter referred to as "Sambrook *et al.*"

Once DNA encoding a sequence of interest is isolated and cloned, one can express the encoded proteins in a variety of recombinantly engineered cells. It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of DNA encoding. No attempt to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes is made here.

In brief summary, the expression of natural or synthetic nucleic acids encoding a sequence of interest will typically be achieved by operably linking the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of polynucleotide sequence of interest. To obtain high level expression of a cloned gene, it is desirable to construct expression plasmids which contain, at the minimum, a strong promoter to direct transcription, a ribosome binding site for translational initiation, and a

transcription/translation terminator. The expression vectors may also comprise generic expression cassettes containing at least one independent terminator sequence, sequences permitting replication of the plasmid in both eukaryotes and prokaryotes, *i.e.*, shuttle vectors, and selection markers for both prokaryotic and eukaryotic systems. *See* Sambrook *et al.*

The E2F-RB fusion constructs of the invention can be introduced into the tissue of interest in vivo or ex vivo by a variety of methods. In some embodiments of the invention, the nucleic acid, preferably DNA, is introduced to cells by such methods as microinjection, calcium phosphate precipitation, liposome fusion, or biolistics. In further embodiments, the DNA is taken up directly by the tissue of interest. In other embodiments, the constructs are packaged into a viral vector system to facilitate introduction into cells.

Viral vector systems useful in the practice of the instant invention include adenovirus, herpesvirus, adeno-associated virus, minute virus of mice (MVM), HIV, sindbis virus, and retroviruses such as Rous sarcoma virus, and MoMLV. Typically, the constructs of the instant invention are inserted into such vectors to allow packaging of the E2F-RB expression construct, typically with accompanying viral DNA, infection of a sensitive host cell, and expression of the E2F-RB gene. A particularly advantageous vector is the adenovirus vector disclosed in Wills, et al. Human Gene Therapy **5:1079-1088** (1994).

In still other embodiments of the invention, the recombinant DNA constructs of the invention are conjugated to a cell receptor ligand for facilitated uptake (e.g., invagination of coated pits and internalization of the endosome) through a DNA linking moiety (Wu, et al. J. Biol. Chem. **263:14621-14624** (1988); WO 92/06180). For example, the DNA constructs of the invention can be linked through a polylysine moiety to asialo-oromucocid, which is a ligand for the asialoglycoprotein receptor of hepatocytes.

Similarly, viral envelopes used for packaging the constructs of the invention can be modified by the addition of

receptor ligands or antibodies specific for a receptor to permit receptor-mediated endocytosis into specific cells (e.g., WO 93/20221, WO 93/14188; WO 94/06923). In some embodiments of the invention, the DNA constructs of the invention are linked to viral proteins, such as adenovirus particles, to facilitate endocytosis (Curiel, et al. Proc. Natl. Acad. Sci. U.S.A. **88:8850-8854** (1991)). In other embodiments, molecular conjugates of the instant invention can include microtubule inhibitors (WO 94/06922); synthetic peptides mimicking influenza virus hemagglutinin (Plank, et al. J. Biol. Chem. **269:12918-12924** (1994)); and nuclear localization signals such as SV40 T antigen (WO 93/19768).

In some embodiments of the invention, the RB polypeptides of the invention are administered directly to a patient in need of treatment. A "therapeutically effective" dose is a dose of polypeptide sufficient to prevent or reduce severity of a hyperproliferative disorder. As used herein, the term "hyperproliferative cells" includes but is not limited to cells having the capacity for autonomous growth, i.e., existing and reproducing independently of normal regulatory mechanisms. Hyperproliferative diseases may be categorized as pathologic, i.e., deviating from normal cells, characterizing for constituting disease, or may be categorized as non-pathologic, i.e., deviation from normal but not associated with a disease state. Pathologic hyperproliferative cells are characteristic of the following disease states: restenosis, diabetic retinopathy, thyroid hyperplasia, Grave's disease, psoriasis, benign prostatic hypertrophy, Li-Fraumeni syndrome including breast cancer, sarcomas and other neoplasms, bladder cancer, colon cancer, lung cancer, various leukemias and lymphomas. Examples of non-pathological hyperproliferative cells are found, for instance, in mammary ductal epithelial cells during development of lactation and also in cells associated with wound repair. Pathological hyperproliferative cells characteristically exhibit loss of contact inhibition and a decline in their ability to selectively adhere which implies a further breakdown in intercellular communication. These

changes include stimulation to divide and the ability to secrete proteolytic enzymes.

The constructs of the invention are useful in the therapy of various cancers and other conditions in which the administration of RB is advantageous, including but not limited to peripheral vascular diseases and diabetic retinopathy. Although any tissue can be targeted for which some tissue-specific expression element, such as a promoter, can be identified, of particular interest is the tissue-specific administration of an RB construct for hyperproliferative disorders such as restenosis, for which the smooth muscle actin promoter is preferable.

The compositions of the invention will be formulated for administration by manners known in the art acceptable for administration to a mammalian subject, preferably a human. In some embodiments of the invention, the compositions of the invention can be administered directly into a tissue by injection or into a blood vessel supplying the tissue of interest. In further embodiments of the invention the compositions of the invention are administered "locoregionally", i.e., intravesically, intralesionally, and/or topically. In other embodiments of the invention, the compositions of the invention are administered systemically by injection, inhalation, suppository, transdermal delivery, etc. In further embodiments of the invention, the compositions are administered through catheters or other devices to allow access to a remote tissue of interest, such as an internal organ. The compositions of the invention can also be administered in depot type devices, implants, or encapsulated formulations to allow slow or sustained release of the compositions.

The invention provides compositions for administration which comprise a solution of the compositions of the invention dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known

sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

The concentration of the compositions of the invention in the pharmaceutical formulations can vary widely, i.e., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

The compositions of the invention may also be administered via liposomes. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. In these preparations the composition of the invention to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to a desired target, such as antibody, or with other therapeutic or immunogenic compositions. Thus, liposomes either filled or decorated with a desired composition of the invention of the invention can delivered systemically, or can be directed to a tissue of interest, where the liposomes then deliver the selected therapeutic/immunogenic peptide compositions.

Liposomes for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, e.g., liposome size, acid lability and stability of the liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al. Ann. Rev. Biophys. Bioeng. 9:467

(1980), U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369, incorporated herein by reference.

A liposome suspension containing a composition of the invention may be administered intravenously, locally, topically, etc. in a dose which varies according to, inter alia, the manner of administration, the composition of the invention being delivered, and the stage of the disease being treated.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more compositions of the invention of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the compositions of the invention are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of compositions of the invention are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, e.g., lecithin for intranasal delivery.

The constructs of the invention can additionally be delivered in a depot-type system, an encapsulated form, or an

implant by techniques well-known in the art. Similarly, the constructs can be delivered via a pump to a tissue of interest.

In some embodiments of the invention, the compositions of the invention are administered ex vivo to cells or tissues explanted from a patient, then returned to the patient. Examples of ex vivo administration of gene therapy constructs include Arteaga et al. Cancer Research **56(5):1098-1103** (1996); Nolta et al. Proc Natl. Acad. Sci. USA **93(6):2414-9** (1996); Koc et al. Seminars in Oncology **23 (1):46-65** (1996); Raper et al. Annals of Surgery **223(2):116-26** (1996); Dalesandro et al. J. Thorac. Cardi. Surg. **11(2):416-22** (1996); and Makarov et al. Proc. Natl. Acad. Sci. USA **93(1):402-6** (1996).

In some embodiments of the invention, the constructs of the invention are administered to a cardiac artery after balloon angioplasty to prevent or reduce the severity of restenosis. The constructs of the invention can be used to coat the device used for angioplasty (see, for example, Willart, et al. Circulation **89:2190-2197** (1994); French, et al. Circulation **90:2402-2413** (1995)). In further embodiments, the fusion polypeptides of the invention can be used in the same manner.

The following examples are included for illustrative purposes and should not be considered to limit the present invention.

EXAMPLES

Example I

E2F-RB Fusions

A. Introduction

In this example, expression plasmids which encode different segments of E2F fused to RB56 polypeptide were constructed. RB56 is a subfragment of full length RB which contains the "pocket" domains necessary for growth suppression (Hiebert, et al. MCB **13:3384-3391** (1993); Qin, et al. Genes

and Dev. 6:953-964 (1992)). E2F194 contains E2F amino acids 95-194. This fragment contains only the DNA binding domain of E2F. E2F286 contains the DNA binding domain and the DP-1 heterodimerization domain. Both E2F fragments lack the N-terminal cyclin A-kinase binding domain, which appears to down-regulate the DNA binding activity of E2F (Krek et al. Cell 83:1149-1158 (1995); Krek et al. Cell 78:161-172 (1994)).

B. Construction of Vectors

Plasmid pCTM contains a CMV promoter, a tripartite adenovirus leader flanked by T7 and SP6 promoters, and a multiple cloning site with a bovine growth hormone (BGH) polyadenylation site and a SV-40 poly adenylation site downstream. A diagrammatic representation of pCTM is provided in Figure 3. The DNA sequence for pCTM is provided in Figure 4.

pCTMI was constructed from pCTM by digesting pCTM with Xho I and Not I and subcloning a 180 bp intron Xhol-Not I fragment from a pCMV-β-gal vector (Clonetech). A diagrammatic representation of pCTMI is provided in Figure 5. The DNA sequence is provided in Figure 6.

pCTMIE was constructed by amplifying the SV40 enhancer from SV40 viral DNA in a polymerase chain reaction. The amplified product was digested with BglII and inserted into BamH1-digested pCMTI and ligated in the presence of BamHI. The plasmid is depicted diagrammatically in Figure 7. The DNA sequence is provided in Figure 8.

pCTM-RB was prepared as follows. A 3.2 KB Xba I - Cla I fragment of pETRBc (Huang et al. Nature 350:160-162 (1991)) containing the full length human RB cDNA was ligated to Xba I-Cla I digested pCTM. pCTM-RB56 was prepared by ligating the digested pCTM to a 1.7 KB Xba 1 -Cla I fragment containing the coding sequence for RB56. pCTMI-RB, pCTMIE-RB, pCTMI-RB56(amino acids 381-928) and pCTMIE-RB56(amino acids 381-928) were all constructed by the same methods.

C. RB-E2F fusion Constructs

Figure 9 depicts the fusion constructs used in these studies. These E2F constructs commenced at amino acid 95 and lacked part of the cyclin A binding domain. E2F437 contained the DNA binding domain (black), heterodimerization domain (white) and transactivation domain (stippled). E2F194 contained solely the DNA binding domain. E2F286 contained the DNA binding domain and DP-1 heterodimerization domain. RB56-5s refers to an RB variant having alanine substitutions at amino acid residues 606, 612, 788, 807 and 811. In E2F194-RB56-5s and E2F286-RB56-5s, the E2F fragments were fused in frame to codon 379 of RB-5s. RB56-C706F contained an inactivating point mutation (Kaye et al. Proc. Natl. Acad. Sci. U.S.A. 87:6922-6926 (1990)).

pCMV-E2F194 and pCMV-E2F437 were constructed as follows. DNA encoding amino acids 95-194 of E2F (containing the DNA binding domain) or amino acids 95-437 was amplified in a polymerase chain reaction, digested with HindII, and ligated into SmaI/HindII digested pCMV-RB56 vectors. pCMVE2F286 was constructed by digesting pCMV-E2F437 with AflII, treating the ends with DNA pol I (Klenow fragment) and religating in the presence of AflII. The blunt end ligation created a stop codon at position 287. pCMV-E2F286-5s was constructed by ligating AflII (blunt)/HindIII digested pE2F437 to a Sal I (blunt)-HindIII fragment containing the RB56-5s coding sequence. pCTMIE-E2F194-5s and pCTMIE-E2F286-RB5s were constructed by ligating EcoRI-EcoRV digested pCTMIE (4.2 KB) to HindIII (blunt)-EcoRI fragments from either pCMV-E2F194-RB5s or pCMV-E2F286-RB5s.

D. Promoter Repression

To measure the effect of the E2F-RB fusion proteins, cervical carcinoma cell line C33A (ATCC # HTB-31) was transfected with equivalent amounts of E2F194-RB56 or E2F RB56 with an E2-CAT reporter plasmid (See, e.g., Weintraub et al. Nature 358:259-261 (1992)).

In the C33A assay, 250,000 C33A cells were seeded into each of well of 6-well tissue culture plates and allowed to adhere overnight. 5 μg each of pCMV-RB56, pCMV-E2F RB56,

or pCMV-E2F plasmid were cotransfected (calcium phosphate method, MBS transfection kit, Stratagene) with 5 μg of indicated reporter construct E2-CAT or SVCAT) and 2.5 μg β-gal plasmid (pCMV-β, Clontech) per well into duplicate wells. Cells were harvested 72 hour after transfection and extracts were prepared.

In the 5637 assay, 250,000 5637 cells were seeded as described above. 1 μg each of RB or E2F-RB fusion plasmid, E2-CAT or SV-CAT reporter plasmid and pCMV-β-galactosidase were cotransfected using the lipofectin reagent (BRL, Bethesda, Maryland) according to the manufacturer's instructions.

CAT assays were performed using either 20 μL (C33A) or 50 μL (5637) of cell extract (Gorman et al. Mol. Cell. Biol. 2:1044 (1982)). TLCs were analyzed on a Phosphoimager SF (Molecular Dynamics). CAT activities were normalized for transfection efficiency according to β-galactosidase activities of each extract. β-galactosidase activities of extracts were assayed as described by Rosenthal et al. (Meth. Enzym. 152:704 (1987)).

The results of these studies were as follows. Transfection of the E2-CAT reporter alone or in the presence of the nonfunctional control RB56-H209 mutant yielded relatively high CAT activity. Cotransfection of wild-type RB56 or the variant RB56-5s resulted in a 10 to 12 fold repression of CAT activity, indicating that RB56 or RB56-5s are both capable of efficiently repressing E2F-dependent transcription. E2F194-RB5s and E2F286-RB5s repressed transcription approximately 50 fold. Transcriptional repression required both the RB56 and the E2F components of the fusion proteins, as expression of E2F194 and E2F286 did not mediate transcriptional repression. No repression of SV40-CAT transcription occurred with E2F-RB constructs, thus demonstrating the specificity of the transcriptional repression by E2FRB for the E2 promoter. These results are depicted diagrammatically in Figure 10.

E. Cell cycle arrest

The ability of E2F-RB fusion polypeptides to cause G1 arrest in Saos-2 (RB-/- cells) (ATCC # HTB-85) and C33A cells was investigated. Previous studies have shown that RB-mediated E2 promoter repression and G1 arrest are linked in Saos-2 cells but dissociated in C33A (RBmut) cells (Xu, et al. PNAS **92:1357-1361** (1992)). Cells were washed in PBS and were fixed in 1 mL -20°C 70% ethanol for 30 minutes. Cells were collected by centrifugation and resuspended in 0.5 mL 2% serum containing 10 μg/ml RNase A and incubated for 30 minutes at 37°C 0.5 mL of PBS containing propidium iodide (100 μg/ml) was added to each sample, mixed and cells were filtered through a FACS tube capstrainer. FACS analysis was performed on a FACS-Scan (Becton-Dickenson) using doublet discrimination. 5,000-10,000 CD20+ events were analyzed. Percent of cells in G_0/G_1, S, and G_2/M was determined using Modfit modeling software.

The results of this experiment were as follows. Both full length RB110 and the truncated version RB56, but not the control mutant RB-H209, caused G_1 arrest in Saos-2 cells (Table 1). Similarly, the RB56-5s, E2F-194-RB56-5s and E2F286-RB56-5s all were capable of arresting cells in G_0/G_1. Transfection of the DNA binding domain, E2F194, did not block S-phase entry in Saos-2 as previously described for rodent cells (Dobrowolski, et al. Oncogene **9:2605-2612** (1994)). In contrast, RB110, RB56, and E2F-RB fusion proteins were not capable of arresting C33A cell lines indicating that the transcriptional repression observed in these cells does not translate into G_1 arrest.

The ability of the E2F-RB fusion proteins to arrest 5637 cells was also investigated (Table 2). RB56 and RB56-5s both efficiently arrested cells in G_0/G_1 (approximately 90% of cells in G_0-G_1), whereas E2F194-RB56-5s and E2F286-RB56-5s are slightly less efficient (about 80% of cells in G_0/G_1) at promoting G_0/G_1 arrest. Without being limited to any one theory, the less efficient arrest of both Saos-2 and 5637 cells by the E2F-RB fusion proteins appears due to the lower levels of steady-state protein produced in these cells (Figure 11, panels b and c).

Table 1: Cell Cycle Regulation by RB and E2F-RB fusion proteins in RBneg cells

	% Cells		
	$CD20^+$ G_0/G_1	G_2/M	S-phase
H209	52.1	27.1	20.8
p56RB	78.8	14.2	7.0
p110RB	70.9	14.9	14.8
p56RB-5s	84.8	13.2	2.0
p56RB-p5	81.3	11.5	7.3
E2F-194-5s	77.8	14.9	7.3
E2F-286-5s	72.2	15.0	12.8
E2F-194	49.9	28.0	22.1

Table 2: Growth Suppression of 5637 Bladder Cells by RB and E2F-RB fusion proteins

5637/$CD20^+$	% Cells		
	G_0/G_1	S	G_2M
CD20	59.7	16.9	20.6
RB56-C706F	57.4	16.9	24.3
RB56WT	90.7	4.12	4.88
RB56-5s	89.91	3.51	6.1
E2F1 94-5s	80.1	1.31	0
E2F-286-5s	79.21	8.1	0

F. Activity of Fusion Proteins in Functional RB Background

The activity of the E2F-RB fusion proteins in a cellular background containing functional RB was then determined. NIH-3T3 cells were transfected with RB56 or E2F-RB56 fusions and stained with anti-RB monoclonal antibody 3C8 (Wen et al. J. Immuno. Meth. **169:231-240** (1994)). FACS analysis was performed of the RB expressing cells. The

results are shown in Figure 12. The non-gated population (g) shows the characteristic cell cycle distribution for NIH-3T3 cells (60% GO, 28% S, 10% G2/M). In contrast, in cells transfected with RB56 (a,b) or E2F-RB fusion proteins (c-f), greater than 90% of the RB-expressing cells were arrested in G_0/G_1. These data demonstrate that the ability of RB and E2F-RB56 fusions to arrest cells in G_0/G_1 is not limited to RB negative tumor cells. The relative levels of protein expressed in transfected NIH-3T3 cells was also investigated. RB110 was not expressed efficiently in these cells.

Thus, these data demonstrate that E2F-RB fusion proteins are more efficient transcriptional repressors than either pRB or RB56 alone, and that RB can repress transcription by remaining bound to E2F rather than directly blocking the transactivation domain of E2F. These data support the use of E2F-RB fusions as RB agonists in both RB+ cells and in RB negative or RB mutant cells.

Example II.

Tissue-Specific Expression of E2F-RB Fusions

A. Construction of Recombinant Adenovirus:

In this experiment, recombinant adenoviruses comprising an RB polypeptide under the control of a CMV or smooth muscle alpha actin promoter were generated.

The smooth muscle α-actin promoter (bases -670 through +5, Reddy et al. "Structure of the Human Smooth Muscle α-Actin Gene." J. Biol. Chem. **265:1683-1687** (1990), Nakano, et al. "Transcriptional Regulatory Elements In The 5' Upstream and First Intron Regions of The Human Smooth Muscle (aortic type) α-Actin-Encoding Gene." Gene **99:285-289** (1991) was isolated by PCR from a genomic library with 5' Xho I and Avr II and 3' Xba I, Cla I and Hind III restriction sites added for cloning purposes. The fragment was subcloned as an Xho 1, Hind III fragment into a plasmid for sequencing to verify base composition. A fusion construct 286-56 containing the DNA and heterodimerization domain of E2F-1 (bases 95-286) linked to p56 (amino acids 379-928 of full length RB) was subcloned as

an Xba I, Cla I fragment directly downstream of the smooth muscle α-actin promoter, and this expression cassette was digested out and cloned into the plasmid pAd/ITR/IX- as an Xba I to AvrII, and Cla I fragment to create the plasmid pASN286-56. This plasmid consisted of the adenovirus type 5 inverted terminal repeat (ITR), packaging signals and Ela enhancer, followed by the human smooth muscle α-actin promoter and 286-56 cassette, and then Ad 2 sequence 4021-10462 (which contains the E1b/protein IX poly A signal) in a pBR322 background. Recombinant adenovirus was produced by standard procedures. The plasmid pASN286-56 was linearized with Ngo MI and co-transfected into 293 cells with the large fragment of Cla I digested rAd34 which has deletions in both the E3 and E4 regions of adenovirus type 5. Ad34 was a serotype 5 derivative with a 1.9 KB deletion in early region 3 resulting from deletion of the Xba I restriction fragment extending from Ad5 coordinates 28593 to 30470 and a 1.4 KB deletion of early region 4 resulting from a Taq 1 fragment of E4 (coordinates 33055-35573) being replaced with a cDNA containing E4 ORF 6 and 6/7.

Recombinant adenovirus produced by homologous recombination was isolated and identified by restriction digest analysis and further purified by limiting dilution. Additional control recombinant adenoviruses are described elsewhere and include the control virus ACN (CMV promoter, Wills, et al. "Gene Therapy For Hepatocellular Carcinoma: Chemosensitivity Conferred By Adenovirus-Mediated Transfer of The HSV-1 Thymidine Kinase Gene." Cancer Gene Therapy **2:191-197** (1995)), and ACN56 (RB expressed under control of a CMV promoter).

ACN56 was prepared as follows. A plasmid containing p56 cDNA was constructed by replacing the p53 cDNA from the plasmid ACNP53 (Wills et al. Human Gene Therapy 5:1079-1088 (1994)) with a 1.7 KB Xba I- BamHI fragment isolated from plasmid pET 9a-Rb56 (Antelman et al. Oncogene 10:697-704 (1995)) which contains p56 cDNA. The resulting plasmid contained amino acids 381-928 of p56, the Ad5 inverted terminal repeat, viral packaging signals and E1a enhancer,

followed by the human cytomegalovirus immediate early promoter (CMV) and Ad 2 tripartite leader cDNA to drive p56 expression. The p56 cDNA was followed by Ad 2 sequence 4021-10462 in a pBR322 background. This plasmid was linearized with EcoRI and cotransfected with the large fragment of bsp 106 digested DL327 (E3 deleted; Thimmappaaya et al. Cell 31:543-551 (1982)) or h5ile4 (E4 deleted; Hemstrom et al. J. Virol. 62:3258-3264 (1988)). Recombinant viruses were further purified by limiting dilution.

B. Cellular Proliferation

In this experiment, cell lines were infected in culture with recombinant adenovirus RB constructs to ascertain the relative expression of the RB polypeptide and the effect on cell proliferation.

For H358 (ATCC # Crl 5807) and MDA-MB468 (ATCC # HTB 132, breast adenocarcinoma) cells, 5,000 cell/well were plated in normal growth media in a 96 well microtiter plate (Costar) and allowed to incubate overnight at 37°C, 7% CO_2. Viruses were serially diluted in growth media and used to infect cells at the indicated doses for 48 hours. At this point, ^{3}H-thymidine was added (Amersham, 0.5 μCi/well) and the cells were incubated at 37°C for another 3 hours prior to harvest. Both A7r5 (ATCC CRL1444, rat smooth muscle) and A10 (ATCC CRL 1476, rat smooth muscle) cells were seeded at 3,000 cells/well in either DME + 0.5% FCS or DME + 20% FCS respectively. Virus was serially diluted in the seeding media and used to infect the cells at the doses indicated in the Figures. The infection and labelling procedure were the same for A10 cells as with the H358 and MDA-MB468 cells except that 2 μCi/well of label was used. The A7r5 cells were not infected with virus until 48 hours after seeding. Forty eight hours after infection, the serum concentration was raised to 10% FCS and 2 μCi/well of ^{3}H-thymidine was added and incubation continued for an additional 3 hours prior to harvest. All cells were harvested by aspirating media from the wells, trypsinization of the cells, and harvesting using a 96 well GF/C filter with

a Packard Top count cell harvester. Results are plotted as the mean percentage (+/- SD) of media treated control proliferation versus dose of virus in Figures 13 and 14.

Thus, Figure 13 depicts a comparison of the effects of adenovirus p56 constructs on muscle cells A10 and A7R5 cells. The CMV-driven p56 (ACN 56) virus inhibited A10 growth to approximately the same extent as the actin promoter-driven E2F-fusion constructs (ASN586-56 #25,26). In Figure 14, the effects of adenovirus constructs on inhibition of a breast cancer cell line, MDA Mβ468 and a non- small cell lung carcinoma cell line, H358, are depicted. In these experiments, actin promoter-driven E2F-p56 was ineffective, while the CMV promoter-driven p56 was effective in inhibiting growth of non-smooth muscle cells.

To determine whether the non-smooth muscle cells were more infectable with adenovirus than the smooth muscle cell lines used, the four cells lines, H358, MB468, A7R5, and A10 were infected at an MOI of 5 with an adenovirus expressing β-galactosidase (ACβGL; Wills, et al. Human Gene Therapy **5:1079-1088** (1994)) and degree of β-gal staining was examined. As shown in Figure 15 (top), the non-smooth muscle cell lines were significantly more infectable than the smooth muscle cell lines. In a further test, cells were infected at higher multiplicities of infection (50, 100, 250, 500) with ACN56 and the amount of p56 present in the infected cells detected by autoradiography. As can be seen in Figure 15 (bottom), the non-muscle cell lines had significantly more p56 present, since as a result of their greater infectivity, infected cells have a greater viral load and thus more copies of the p56 template driven by the non-tissue specific CMV promoter.

In a further experiment, the specificity of the actin smooth muscle promoter for smooth muscle tissue was ascertained. In this experiment, β-gal expression levels in cells infected with β-gal constructs driven with different promoters were measured. As can be seen in Figure 19, despite the lower infectivity of the smooth muscle cells, expression was only evident in these cells using the smooth muscle alpha actin promoter.

Figure 21 depicts a comparison of the effects of a CMV driven p56 recombinant adenovirus (ACN56E4) vs a human smooth muscle alpha-actin promoter driven E2F-p56 fusion construct (ASN286-56) vs control adenoviral construct containing either the CMV or smooth muscle alpha-actin promoters without a downstream transgene (ACNE3 or ASBE3-2 isolates shown, respectively). Assays were 3H-thymidine uptake either in a smooth muscle cell line (A7R5) or a non-muscle cell line (MDA-MB468, breast carcinoma). Results demonstrated muscle tissue specificity using the smooth muscle alpha-actin promoter and specific inhibition of both the p56 and E2F-p56 transgenes relative to their respective controls.

C. Inhibition of Restenosis

The model of balloon injury was based on that described by Clowes, et al. (Clowes, Lab. Invest. 49:327-333 (1983)). Male Sprague-Dawley rats weighing 400-500g were anesthetized with an intraperitoneal injection of sodium pentobarbital (45 mg/kg. Abbot Laboratories, North Chicago, Illinois). The bifurcation of the left common carotid artery was exposed through a midline incision and the left common, internal, and external carotid arteries were temporarily ligated. A 2F embolectomy catheter (Baxter Edwards Healthcare Corp., Irvine, CA) was introduced into the external carotid and advanced to the distal ligation of the common carotid. The balloon was inflated with saline and drawn towards the arteriotomy site 3 times to produce a distending, deendothelializing injury. the catheter was then withdrawn. Adenovirus (1 x 10^9 pfu of Ad-RB (ACNRb) or Ad-p56 (ACN56) in a volume of 10μl diluted to 100μl with 15% (wt/vol) Poloxamer 407 (BASF, Parsippany, N.J.) or Ad-β-Gal (1 x 10^9 pfu, diluted as above) was injected via a canula, inserted just proximal to the carotid bifurcation into a temporarily isolated segment of the artery. The adenovirus solution was incubated for 20 minutes after which the viral infusion was withdrawn and the cannula removed. The proximal external carotid artery was then ligated and blood flow was restored to the common carotid artery by release of the ligatures. The experimental protocol

was approved by the Institutional Animal Care and Use Committee and complied with the "Guide for the Care and Use of Laboratory Animals." (NIH Publication No. 86-23, revised 1985).

Rats were sacrificed at 14 days following treatment with an intraperitoneal injection of pentobarbital (100 mg/kg.). The initially balloon injured segment of the left common carotid artery, from the proximal edge of the omohyoid muscle to the carotid bifurcation, was perfused with saline and dissected free of the surrounding tissue. The tissue was fixed in 100% methanol until imbedded in paraffin. Several 4-μm sections were cut from each tissue specimen. One section from each specimen was stained with hematoxylin and eosin and another with Richardson's combination elastic-trichrome stain conventional light microscopic analysis.

Histological images of cross sections of hematoxylin and eosin or elastic-trichrome stained arterial sections were projected onto a digitizing board (Summagraphics) and the intimal, medial and luminal areas were measured by quantitative morphometric analysis using a computerized sketching program (MACMEASURE, version 1.9, National Institute of Mental Health).

Results were expressed as the mean ± S.E.M. Differences between groups were analyzed using an unpaired two-tailed Student's *t* test. Statistical significance was assumed when the probability of a null effect was <0.05.

Results are shown in Figures 17 and 18. In Figure 17, the relative inhibition of neointima formation is depicted graphically, demonstrating the ability of p56 and RB to inhibit neointima formation. Figure 18 provides photographic evidence of the dramatic reduction of neointima in the presence of p56.

Adenovirus-treated carotid arteries were harvested from rats at 2 days following balloon injury and infections. Tissue was fixed in phosphate-buffered formalin until embedded in paraffin. Tissue was cut into 4μm cross-sections and dewaxed through xylene and graded alcohols. Endogenous peroxidase was quenched with 1% hydrogen peroxide for 30

minutes. Antigen retrieval was performed in 10mM sodium citrate buffer, pH 6.0 at 95°C for 10 minutes. A monoclonal anti-RB antibody (AB-5, Oncogene Sciences, Uniondale, New York) was applied 10μg/ml in PBS in a humid chamber at 4°C for 24 hours. Secondary antibody was applied from the Unitect Mouse Immunohistochemistry Kit (Oncogene Sciences, Uniondale, New York) according to the manufacturer's instructions. The antibody complexes were visualized using 3,3'-diaminobenzidene (DAB, Vector Laboratories, Burlingame, CA). Slides were thin counterstained with hematoxylin and mounted. The results are depicted in Figure 20.

All references cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. A polypeptide comprising a fusion of a transcription factor, the transcription factor comprising a DNA binding domain, and a retinoblastoma (RB) polypeptide, the RB polypeptide comprising a growth suppression domain.

2. A nucleic acid encoding the fusion polypeptide of claim 1.

3. The nucleic acid of claim 2, wherein the nucleic acid in inserted in an adenovirus vector.

4. The polypeptide of claim 1, wherein the transcription factor is E2F.

5. The polypeptide of claim 4, wherein the cyclin A binding domain of the E2F is deleted or nonfunctional.

6. The polypeptide of claim 1, wherein the retinoblastoma polypeptide is RB56.

7. The polypeptide of claim 1, wherein the retinoblastoma polypeptide is wild type RB.

8. The polypeptide of claim 1, wherein the retinoblastoma polypeptide comprises from about amino acid residue 379 to about amino acid residue 928 of pRB.

9. The polypeptide of claim 1, wherein the retinoblastoma polypeptide comprises at least one substitution of amino acid residues selected from the group consisting of 2, 608, 612, 788, 807, and 811 of pRB.

10. The polypeptide of claim 5, wherein the E2F comprises about amino acid residues 95 to about 286.

11. The polypeptide of claim 4, wherein the E2F comprises about amino acid residues 95 to about 194.

12. The polypeptide of claim 1, wherein the fusion comprises EF2 amino acid residues from about 95 to about 194 operatively linked to RB amino acid residues from about 379 to about 928.

13. An expression vector comprising DNA encoding a polypeptide, the polypeptide comprising a fusion of a transcription factor, the transcription factor comprising a DNA binding domain, and a retinoblastoma (RB) polypeptide, the RB polypeptide comprising a growth suppression domain.

14. The vector of claim 13, comprising a tissue-specific promoter operatively linked to DNA encoding the fusion.

15. The vector of claim 14, wherein the tissue specific promoter is a smooth muscle actin promoter.

16. A method for treatment of a hyperproliferative disorder in a patient comprising administering to a patient a therapeutically effective dose of a fusion polypeptide comprising a fusion of a transcription factor, the transcription factor comprising a DNA binding domain, and a retinoblastoma (RB) polypeptide, the RB polypeptide comprising a growth suppression domain.

17. The method of claim 16, wherein the fusion protein is encoded by a nucleic acid delivered to the patient.

18. The method of claim 16, wherein the transcription factor is E2F.

19. The method of claim 18, wherein the cyclin A binding domain of the E2F is deleted or nonfunctional.

20. The method of claim 16, wherein the RB is RB56.

21. The method of claim 16, wherein the RB is wild type RB56.

22. The method of claim 16, wherein the RB comprises from about amino acid residue 379 to about amino acid residue 928.

23. The method of claim 16, wherein the RB comprises at least one substitution of amino acid residues selected from the group consisting of 2, 608, 612, 788, 807, and 811.

24. The method of claim 18, wherein the E2F comprises about amino acid residues 95 to about 286.

25. The method of claim 18, wherein the E2F comprises about amino acid residues 95 to about 194.

26. The method of claim 16, wherein the fusion comprises EF2 amino acid residues from about 95 to about 194 operatively linked to RB amino acid residues from about 379 to about 928.

27. The method of claim 18, wherein the E2F -RB fusion polypeptide is expressed under the control of a tissue-specific promoter.

28. The method of claim 27, wherein the tissue specific promoter is a smooth muscle actin promoter.

29. The method of claim 16, wherein the hyperproliferative disorder is cancer.

30. The method of claim 29, wherein the cancer is bladder cancer.

31. The method of claim 29, wherein the hyperproliferative disorder is restenosis.

32. The method of claim 31, wherein the E2F-RB fusion polypeptide is administered after angioplasty.

33. The method of claim 32, wherein the E2F-RB fusion polypeptide is administered as a coating on an angioplasty device.

34. The method of claim 17, wherein the nucleic acid is administered after angioplasty.

35. The method of claim 17, wherein the nucleic acid is administered as a coating on an angioplasty device.

36. The method of claim 17, wherein the nucleic acid is inserted in an adenovirus vector.

TISSUE SPECIFIC EXPRESSION OF RETINOBLASTOMA PROTEIN

ABSTRACT OF THE DISCLOSURE

Fusions of the transcription factor E2F and the retinoblastoma protein RB are provided, along with methods of treatment of hyperproliferative diseases.

```
        10         20         30         40         50         60
MALAGAPAGG PCAPALEALL GAGALRLLDS SQIVIISAAQ DASAPPAPTG PAAPAAGPCD

        70         80         90        100        110        120
PDLLLFATPQ APRPTPSAPR PALGRPPVKR RLDLETDHQY LAESSGPARG RGRHPGKGVK

       130        140        150        160        170        180
SPGEKSRYET SLNLTTKRFL ELLSHSADGV VDLNWAAEVL KVQKRRIYDI TNVLEGIQLI

       190        200        210        220        230        240
AKKSKNHIQW LGSHTTVGVG GRLEGLTQDL RQLQESEQQL DHLMNICTTQ LRLLSEDTDS

       250        260        270        280        290        300
QRLAYVTCQD LRSIADPAEQ MVMVIKAPPE TQLQAVDSSE NFQISLKSKQ GPIDVFLCPE

       310        320        330        340        350        360
ETVGGISPGK TPSQEVTSEE ENRATDSATI VSPPPSSPPS SLTTDPSQSL LSLEQEPLLS

       370        380        390        400        410        420
RMGSLRAPVD EDRLSPLVAA DSLLEHVRED FSGLLPEEFI SLSPPHEALD YHFGLEEGEG

       430        440        450        460        470        480
IRDLFDCDFG DLTPLDF*.. .......... .......... .......... ..........
```

FIG. 1A

```
        10          20          30          40          50          60
GGAATTCCGT GGCCGGGACT TTGCAGGCAG CGGCGGCCGG GGGCGGAGCG GGATCGAGCC

        70          80          90         100         110         120
CTCGCCGAGG CCTGCCGCCA TGGGCCCGCG CCGCCGCCGC CGCCTGTCAC CCGGGCCGCG

       130         140         150         160         170         180
CGGGCCGTGA GCGTCATGGC CTTGGCCGGG GCCCCTGCGG GCGGCCCATG CGCGCCGGCG

       190         200         210         220         230         240
CTGGAGGCCC TGCTCGGGGC CGGCGCGCTG CGGCTGCTCG ACTCCTCGCA GATCGTCATC

       250         260         270         280         290         300
ATCTCCGCCG CGCAGGACGC CAGCGCCCCG CCGGCTCCCA CCGGCCCCGC GGCGCCCGCC

       310         320         330         340         350         360
GCCGGCCCCT GCGACCCTGA CCTGCTGCTC TTCGCCACAC CGCAGGCGCC CCGGCCCACA

       370         380         390         400         410         420
CCCAGTGCGC CGCGGCCCGC GCTCGGCCGC CCGCCGGTGA AGCGGAGGCT GGACCTGGAA

       430         440         450         460         470         480
ACTGACCATC AGTACCTGGC CGAGAGCAGT GGGCCAGCTC GGGGCAGAGG CCGCCATCCA

       490         500         510         520         530         540
GGAAAAGGTG TGAAATCCCC GGGGGAGAAG TCACGCTATG AGACCTCACT GAATCTGACC

       550         560         570         580         590         600
ACCAAGCGCT TCCTGGAGCT GCTGAGCCAC TCGGCTGACG GTGTCGTCGA CCTGAACTGG

       610         620         630         640         650         660
GCTGCCGAGG TGCTGAAGGT GCAGAAGCGG CGCATCTATG ACATCACCAA CGTCCTTGAG

       670         680         690         700         710         720
GGCATCCAGC TCATTGCCAA GAAGTCCAAG AACCACATCC AGTGGCTGGG CAGCCACACC

       730         740         750         760         770         780
ACAGTGGGCG TCGGCGGACG GCTTGAGGGG TTGACCCAGG ACCTCCGACA GCTGCAGGAG

       790         800         810         820         830         840
AGCGAGCAGC AGCTGGACCA CCTGATGAAT ATCTGTACTA CGCAGCTGCG CCTGCTCTCC

       850         860         870         880         890         900
GAGGACACTG ACAGCCAGCG CCTGGCCTAC GTGACGTGTC AGGACCTTCG TAGCATTGCA

       910         920         930         940         950         960
GACCCTGCAG AGCAGATGGT TATGGTGATC AAAGCCCCTC CTGAGACCCA GCTCCAAGCC

       970         980         990        1000        1010        1020
GTGGACTCTT CGGAGAACTT TCAGATCTCC CTTAAGAGCA AACAAGGCCC GATCGATGTT

      1030        1040        1050        1060        1070        1080
TTCCTGTGCC CTGAGGAGAC CGTAGGTGGG ATCAGCCCTG GGAAGACCCC ATCCCAGGAG

      1090        1100        1110        1120        1130        1140
GTCACTTCTG AGGAGGAGAA CAGGGCCACT GACTCTGCCA CCATAGTGTC ACCACCACCA

      1150        1160        1170        1180        1190        1200
TCATCTCCCC CCTCATCCCT CACCACAGAT CCCAGCCAGT CTCTACTCAG CCTGGAGCAA

      1210        1220        1230        1240        1250        1260
GAACCGCTGT TGTCCCGGAT GGGCAGCCTG CGGGCTCCCG TGGACGAGGA CCGCCTGTCC
```

FIG. 1B

```
      1270       1280       1290       1300       1310       1320
CCGCTGGTGG CGGCCGACTC GCTCCTGGAG CATGTGCGGG AGGACTTCTC CGGCCTCCTC

      1330       1340       1350       1360       1370       1380
CCTGAGGAGT TCATCAGCCT TTCCCCACCC CACGAGGCCC TCGACTACCA CTTCGGCCTC

      1390       1400       1410       1420       1430       1440
GAGGAGGGCG AGGGCATCAG AGACCTCTTC GACTGTGACT TTGGGGACCT CACCCCCCTG

      1450       1460       1470       1480       1490       1500
GATTTCTGAC AGGGCTTGGA GGGACCAGGG TTTCCAGAGT AGCTCACCTT GTCTCTGCAG

      1510       1520       1530       1540       1550       1560
CCCTGGAGCC CCCTGTCCCT GGCCGTCCTC CCAGCCTGTT TGGAAACATT TAATTTATAC

      1570       1580       1590       1600       1610       1620
CCCTCTCCTC TGTCTCCAGA AGCTTCTAGC TCTGGGGTCT GGCTACCGCT AGGAGGCTGA

      1630       1640       1650       1660       1670       1680
GCAAGCCAGG AAGGGAAGGA GTCTGTGTGG TGTGTATGTG CATGCAGCCT ACACCCACAC

      1690       1700       1710       1720       1730       1740
GTGTGTACCG GGGGTGAATG TGTGTGAGCA TGTGTGTGTG CATGTACCGG GGAATGAAGG

      1750       1760       1770       1780       1790       1800
TGAACATACA CCTCTGTGTG TGCACTGCAG ACACGCCCCA GTGTGTCCAC ATGTGTGTGC

      1810       1820       1830       1840       1850       1860
ATGAGTCCAT CTCTGCGCGT GGGGGGGCTC TAACTGCACT TTCGGCCCTT TTGCTCGTGG

      1870       1880       1890       1900       1910       1920
GGTCCCACAA GGCCCAGGGC AGTGCCTGCT CCCAGAATCT GGTGCTCTGA CCAGGCCAGG

      1930       1940       1950       1960       1970       1980
TGGGGAGGCT TTGGCTGGCT GGGCGTGTAG GACGGTGAGA GCACTTCTGT CTTAAAGGTT

      1990       2000       2010       2020       2030       2040
TTTTCTGATT GAAGCTTTAA TGGAGCGTTA TTTATTTATC GAGGCCTCTT TGGTGAGCCT

      2050       2060       2070       2080       2090       2100
GGGGAATCAG CAAAAGGGGA GGAGGGGTGT GGGGTTGATA CCCCAACTCC CTCTACCCTT

      2110       2120       2130       2140       2150       2160
GAGCAAGGGC AGGGGTCCCT GAGCTGTTCT TCTGCCCCAT ACTGAAGGAA CTGAGGCCTG

      2170       2180       2190       2200       2210       2220
GGTGATTTAT TTATTGGGAA AGTGAGGGAG GGAGACAGAC TGACTGACAG CCATGGGTGG

      2230       2240       2250       2260       2270       2280
TCAGATGGTG GGGTGGGCCC TCTCCAGGGG GCCAGTTCAG GGCCCAGCTG CCCCCCAGGA

      2290       2300       2310       2320       2330       2340
TGGATATGAG ATGGGAGAGG TGAGTGGGGG ACCTTCACTG ATGTGGGCAG GAGGGGTGGT

      2350       2360       2370       2380       2390       2400
GAAGGCCTCC CCCAGCCCAG ACCCTGTGGT CCCTCCTGCA GTGTCTGAAG CGCCTGCCTC

      2410       2420       2430       2440       2450       2460
CCCACTGCTC TGCCCCACCC TCCAATCTGC ACTTTGATTT GCTTCCTAAC AGCTCTGTTC

      2470       2480       2490       2500       2520       2520
CCTCCTGCTT TGGTTTTAAT AAATATTTTG ATGACGTTAA AAAAAGGAAT TCGATAT
```

FIG. 1B
(CONTINUED)

```
   1 ttccggtttt tctcagggga cgttgaaatt atttttgtaa cgggagtcgg gagaggacgg
  61 ggcgtgcccc gcgtgcgcgc gcgtcgtcct ccccggcgct cctccacagc tcgctggctc
 121 ccgccgcgga aaggcgtcat gccgcccaaa acccccccgaa aaacggccgc caccgccgcc
 181 gctgccgccg cggaaccccc ggcaccgccg ccgccgcccc ctcctgagga ggacccagag
 241 caggacagcg gcccggagga cctgcctctc gtcaggcttg agtttgaaga aacagaagaa
 301 cctgatttta ctgcattatg tcagaaatta aagataccag atcatgtcag agagagagct
 361 tggttaactt gggagaaagt ttcatctgtg gatggagtat tgggaggtta tattcaaaag
 421 aaaaaggaac tgtggggaat ctgtatcttt attgcagcag ttgacctaga tgagatgtcg
 481 ttcactttta ctgagctaca gaaaaacata gaaatcagtg tccataaatt ctttaactta
 541 ctaaaagaaa ttgataccag taccaaagtt gataatgcta tgtcaagact gttgaagaag
 601 tatgatgtat tgtttgcact cttcagcaaa ttggaaagga catgtgaact tatatatttg
 661 acacaaccca gcagttcgat atctactgaa ataaattctg cattggtgct aaaagtttct
 721 tggatcacat ttttattagc taaaggggaa gtattacaaa tggaagatga tctggtgatt
 781 tcatttcagt taatgctatg tgtccttgac tattttatta aactctcacc tcccatgttg
 841 ctcaaagaac catataaaac agctgttata cccattaatg gttcacctcg aacacccagg
 901 cgaggtcaga acaggagtgc acggatagca aaacaactag aaaatgatac aagaattatt
 961 gaagttctct gtaaagaaca tgaatgtaat atagatgagg tgaaaaatgt ttatttcaaa
1021 aattttatac cttttatgaa ttctcttgga cttgtaacat ctaatggact tccagaggtt
1081 gaaaatcttt ctaaacgata cgaagaaatt tatcttaaaa ataaagatct agatgcaaga
1141 ttatttttgg atcatgataa aactcttcag actgattcta tagacagttt tgaaacacag
1201 agaacaccac gaaaaagtaa ccttgatgaa gaggtgaatg taattcctcc acacactcca
1261 gttaggactg ttatgaacac tatccaacaa ttaatgatga ttttaaattc agcaagtgat
1321 caaccttcag aaaatctgat ttcctatttt aacaactgca cagtgaatcc aaaagaaagt
1381 atactgaaaa gagtgaagga tataggatac atctttaaag agaaatttgc taaagctgtg
1441 ggacagggtt gtgtcgaaat tggatcacag cgatacaaac ttggagttcg cttgtattac
1501 cgagtaatgg aatccatgct taaatcagaa gaagaacgat tatccattca aaattttagc
1561 aaacttctga atgacaacat ttttcatatg tctttattgg cgtgcgctct tgaggttgta
1621 atggccacat atagcagaag tacatctcag aatcttgatt ctggaacaga tttgtctttc
1681 ccatggattc tgaatgtgct taatttaaaa gcctttgatt tttacaaagt gatcgaaagt
1741 tttatcaaag cagaaggcaa cttgacaaga gaaatgataa aacatttaga acgatgtgaa
1801 catcgaatca tggaatccct tgcatggctc tcagattcac ctttatttga tcttattaaa
1861 caatcaaagg accgagaagg accaactgat caccttgaat ctgcttgtcc tcttaatctt
1921 cctctccaga ataatcacac tgcagcagat atgtatcttt ctcctgtaag atctccaaag
1981 aaaaaaggtt caactacgcg tgtaaattct actgcaaatg cagagacaca agcaacctca
2041 gccttccaga cccagaagcc attgaaatct acctctcttt cactgtttta taaaaaagtg
2101 tatcggctag cctatctccg gctaaataca ctttgtgaac gccttctgtc tgagcaccca
2161 gaattagaac atatcatctg gacccttttc cagcacaccc tgcagaatga gtatgaactc
2221 atgagagaca ggcatttgga ccaaattatg atgtgttcca tgtatggcat atgcaaagtg
2281 aagaatatag accttaaatt caaaatcatt gtaacagcat acaaggatct tcctcatgct
2341 gttcaggaga cattcaaacg tgttttgatc aaagaagagg agtatgattc tattatagta
2401 ttctataact cggtcttcat gcagagactg aaaacaaata ttttgcagta tgcttccacc
2461 aggccccta ccttgtcacc aatacctcac attcctcgaa gcccttacaa gtttcctagt
2521 tcacccttac ggattcctgg agggaacatc tatatttcac ccctgaagag tccatataaa
2581 atttcagaag gtctgccaac accaacaaaa atgactccaa gatcaagaat cttagtatca
2641 attggtgaat cattcgggac ttctgagaag ttccagaaaa taaatcagat ggtatgtaac
2701 agcgaccgtg tgctcaaaag aagtgctgaa ggaagcaacc ctcctaaacc actgaaaaaa
2761 ctacgctttg atattgaagg atcagatgaa gcagatggaa gtaaacatct cccaggagag
2821 tccaaatttc agcagaaact ggcagaaatg acttctactc gaacacgaat gcaaaagcag
2941 aaaatgaatg atagcatgga tacctcaaac aaggaagaga aatgaggatc tcaggacctt
2941 ggtggacact gtgtacacct ctggattcat tgtctctcac agatgtgact gtat
```

FIG. 2A

"MPPKTPRKTAATAAAAAAEPPAPPPPPPPEEDPEQDSGPEDLPL
VRLEFEETEEPDFTALCQKLKIPDHVRERAWLTWEKVSSVDGVLGGYIQKKKELWGIC
IFIAAVDLDEMSFTFTELQKNIEISVHKFFNLLKEIDTSTKVDNAMSRLLKKYDVLFA
LFSKLERTCELIYLTQPSSSISTEINSALVLKVSWITFLLAKGEVLQMEDDLVISFQL
MLCVLDYFIKLSPPMLLKEPYKTAVIPINGSPRTPRRGQNRSARIAKQLENDTRIIEV
LCKEHECNIDEVKNVYFKNFIPFMNSLGLVTSNGLPEVENLSKRYEEIYLKNKDLDAR
LFLDHDKTLQTDSIDSFETQRTPRKSNLDEEVNVIPPHTPVRTVMNTIQQLMMILNSA
SDQPSENLISYFNNCTVNPKESILKRVKDIGYIFKEKFAKAVGQGCVEIGSQRYKLGV
RLYYRVMESMLKSEEERLSIQNFSKLLNDNIFHMSLLACALEVVMATYSRSTSQNLDS
GTDLSFPWILNVLNLKAFDFYKVIESFIKAEGNLTREMIKHLERCEHRIMESLAWLSD
SPLFDLIKQSKDREGPTDHLESACPLNLPLQNNHTAADMYLSPVRSPKKKGSTTRVNS
TANAETQATSAFQTQKPLKSTSLSLFYKKVYRLAYLRLNTLCERLLSEHPELEHIIWT
LFQHTLQNEYELMRDRHLDQIMMCSMYGICKVKNIDLKFKIIVTAYKDLPHAVQETFK
RVLIKEEEYDSIIVFYNSVFMQRLKTNILQYASTRPPTLSPIPHIPRSPYKFPSSPLR
IPGGNIYISPLKSPYKISEGLPTPTKMTPRSRILVSIGESFGTSEKFQKINQMVCNSD
RVLKRSAEGSNPPKPLKKLRFDIEGSDEADGSKHLPGESKFQQKLAEMTSTRTRMQKQ
KMNDSMDTSNKEEK"

FIG. 2B

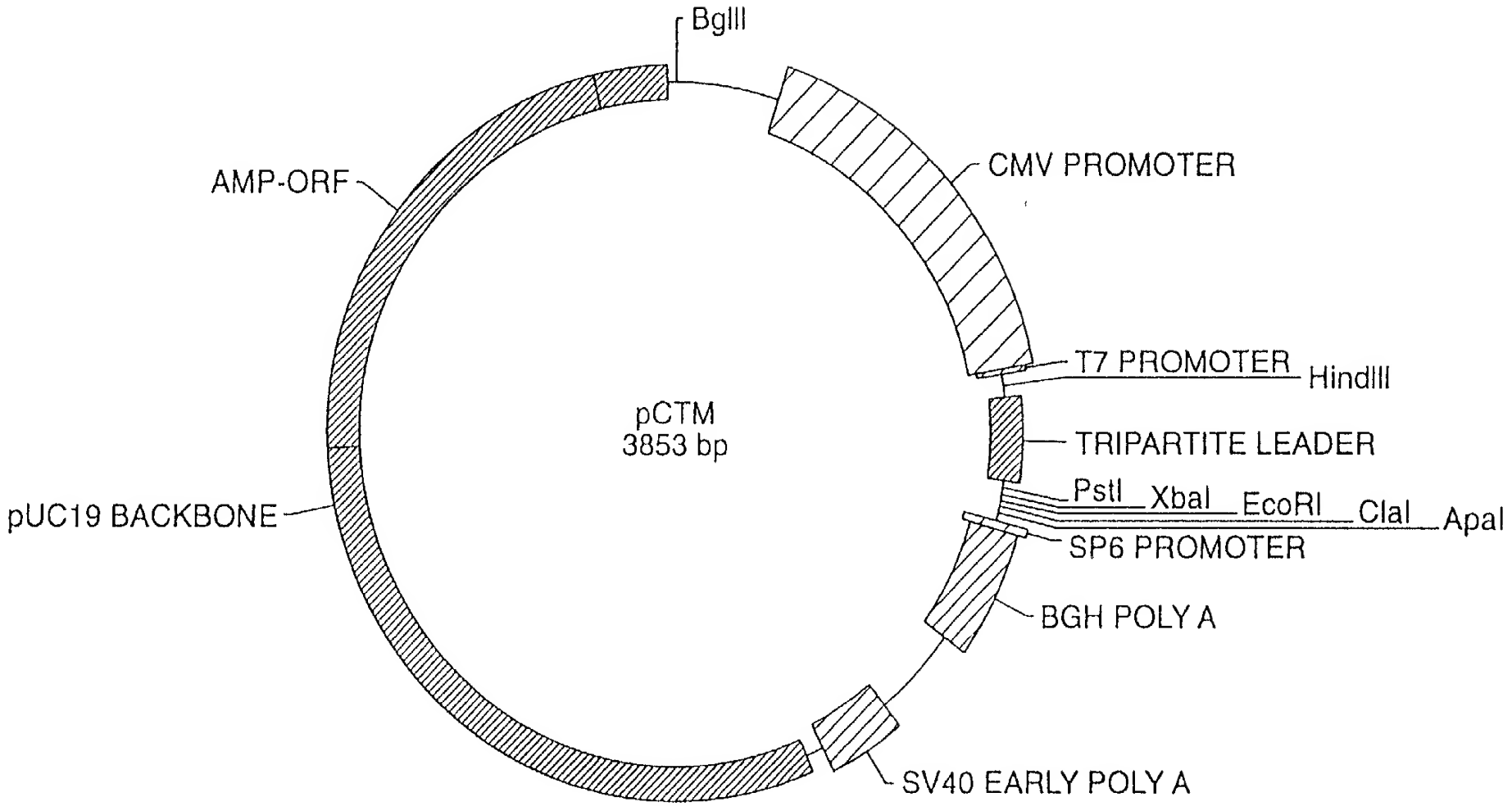

BglII
AMP-ORF
CMV PROMOTER
T7 PROMOTER
HindIII
pCTM
3853 bp
TRIPARTITE LEADER
PstI
XbaI
EcoRI
ClaI
ApaI
pUC19 BACKBONE
SP6 PROMOTER
BGH POLY A
SV40 EARLY POLY A

FIG. 3

```
                               >HincII
                                  |
                               >AccI
                                 ||
          >BglII              >SalI
              |                 |||
        10    |     20        30 |||     40        50        60
     *   *    |  *   *    *   *  |||*    *    *    *    *    *
GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG

                 >AlwNI
                    |
        70          80        90       100       110       120
     *   *    *    *    *    *    *    *    *    *    *    *
CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG

     >ApoI                                  >MfeI
        |                                      |
        |130        140       150       160    |     170       180
     *  |  *    *    *    *    *    *    *     |  *    *    *    *
CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC

                                                           >HincII
                                                                |
                                                     >AflIII    |
                                                         |      |
                                   >NruI               >MluI    |
                                      |                  |      |
       190       200                  |210       220     |230   |
     *   *    *    *    *             |  *    *    *    *|   *  |*
TTAGGGTTAG GCGTTTTGCG CTGCTTCG CGA TGT ACG GGC CAG ATA TAC GCG TTG
                               Arg Cys Thr Gly Gln Ile Tyr Ala Leu>
                               ___d___d___CMV PROMOTER____d___d___>

                 >SpeI      >AseI
                    |          |
      240          250         | 260           270         280
       *     *      |*     *   |  *     *       *     *     *
  ACA TTG ATT ATT GAC TAG TTA TTA ATA GTA ATC AAT TAC GGG GTC ATT
  Thr Leu Ile Ile Asp *** Leu Leu Ile Val Ile Asn Tyr Gly Val Ile>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d___d___>

         290           300          310           320         330
    *     *     *     *     *     *     *     *     *     *     *
  AGT TCA TAG CCC ATA TAT GGA GTT CCG CGT TAC ATA ACT TAC GGT AAA
  Ser Ser *** Pro Ile Tyr Gly Val Pro Arg Tyr Ile Thr Tyr Gly Lys>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d___d___>

        >BglI                                            >AatII
           |                                                |
          340          350         360           370        |
      *     *     *     *     *     *     *     *     *     *
  TGG CCC GCC TGG CTG ACC GCC CAA CGA CCC CCG CCC ATT GAC GTC AAT
  Trp Pro Ala Trp Leu Thr Ala Gln Arg Pro Pro Pro Ile Asp Val Asn>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d___d___>

380         390          400          410          420
 *     *     *     *     *     *     *     *     *     *
  AAT GAC GTA TGT TCC CAT AGT AAC GCC AAT AGG GAC TTT CCA TTG ACG
  Asn Asp Val Cys Ser His Ser Asn Ala Asn Arg Asp Phe Pro Leu Thr>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d___d___>
```

FIG. 4

```
>AatII                                                    >BglI
  |                                                         |
  430           440           450           460           |       470
  | *       *       *       *       *       *       *     |   *       *       *
  TCA ATG GGT GGA CTA TTT ACG GTA AAC TGC CCA CTT GGC AGT ACA TCA
  Ser Met Gly Gly Leu Phe Thr Val Asn Cys Pro Leu Gly Ser Thr Ser>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d___d___>

          >NdeI                                             >AatII
            |                                                 |
     480    |      490           500           510           |       520
       *    | *     *       *       *       *       *       |   *       *
  AGT GTA TCA TAT GCC AAG TAC GCC CCC TAT TGA CGT CAA TGA CGG TAA
  Ser Val Ser Tyr Ala Lys Tyr Ala Pro Tyr *** Arg Gln *** Arg ***>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d___d___>

            >BglI
              |
        530   |       540           550           560           570
  *       *   | *     *       *       *       *       *       *       *
  ATG GCC CGC CTG GCA TTA TGC CCA GTA CAT GAC CTT ATG GGA CTT TCC
  Met Ala Arg Leu Ala Leu Cys Pro Val His Asp Leu Met Gly Leu Ser>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d___d___>

                          >BsaAI                    >NcoI
                            |                         |
                          >SnaBI                    >StyI    >MslI
                            |                         |       |
          580           590           600           610       |
        *       *       *       *       *       *       *   |
  TAC TTG GCA GTA CAT CTA CGT ATT AGT CAT CGC TAT TAC CAT GGT GAT
  Tyr Leu Ala Val His Leu Arg Ile Ser His Arg Tyr Tyr His Gly Asp>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d___d___>

620           630           640           650           660
  *       *       *       *       *       *       *       *       *
  GCG GTT TTG GCA GTA CAT CAA TGG GCG TGG ATA GCG GTT TGA CTC ACG
  Ala Val Leu Ala Val His Gln Trp Ala Trp Ile Ala Val *** Leu Thr>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d___d___>

                                                >AatII                  >BanI
                                                  |                       |
  670           680           690               | 700           710     |
    *       *       *       *       *       *   |   *       *       *   |   *
  GGG ATT TCC AAG TCT CCA CCC CAT TGA CGT CAA TGG GAG TTT GTT TTG
  Gly Ile Ser Lys Ser Pro Pro His *** Arg Gln Trp Glu Phe Val Leu>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d___d___>

     720           730           740           750           760
       *       *       *       *       *       *       *       *
  GCA CCA AAA TCA ACG GGA CTT TCC AAA ATG TCG TAA CAA CTC CGC CCC
  Ala Pro Lys Ser Thr Gly Leu Ser Lys Met Ser *** Gln Leu Arg Pro>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d__-d__->

        770           780           790           800           810
  *       *       *       *       *       *       *       *       *
  ATT GAC GCA AAT GGG CGG TAG GCG TGT ACG GTG GGA GGT CTA TAT AAG
  Ile Asp Ala Asn Gly Arg *** Ala Cys Thr Val Gly Gly Leu Tyr Lys>
  ___d___d___d___d___d___d_CMV PROMOTER__d___d___d___d___d___d___>
```

FIG. 4
(CONTINUED)

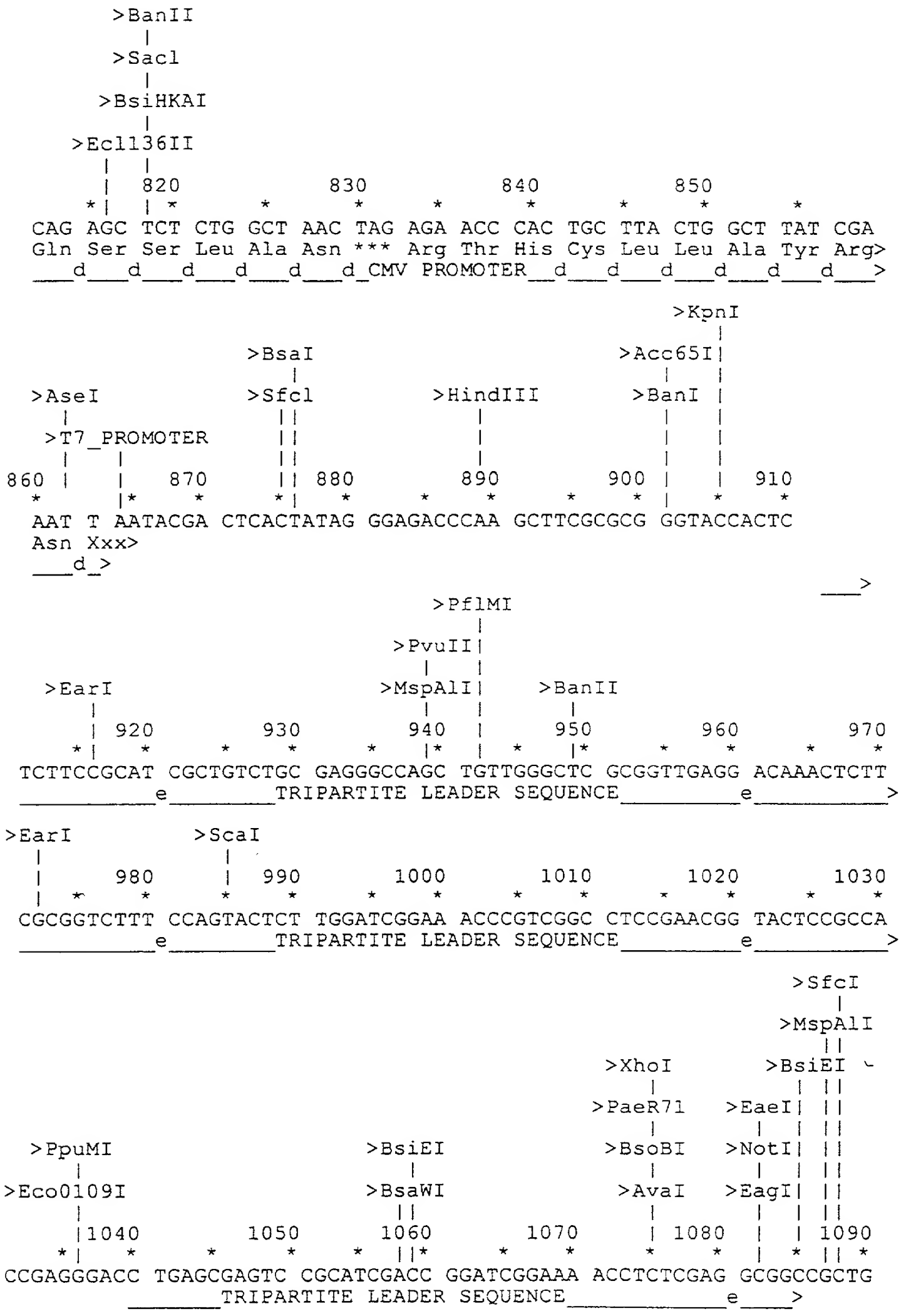


FIG. 4
(CONTINUED)

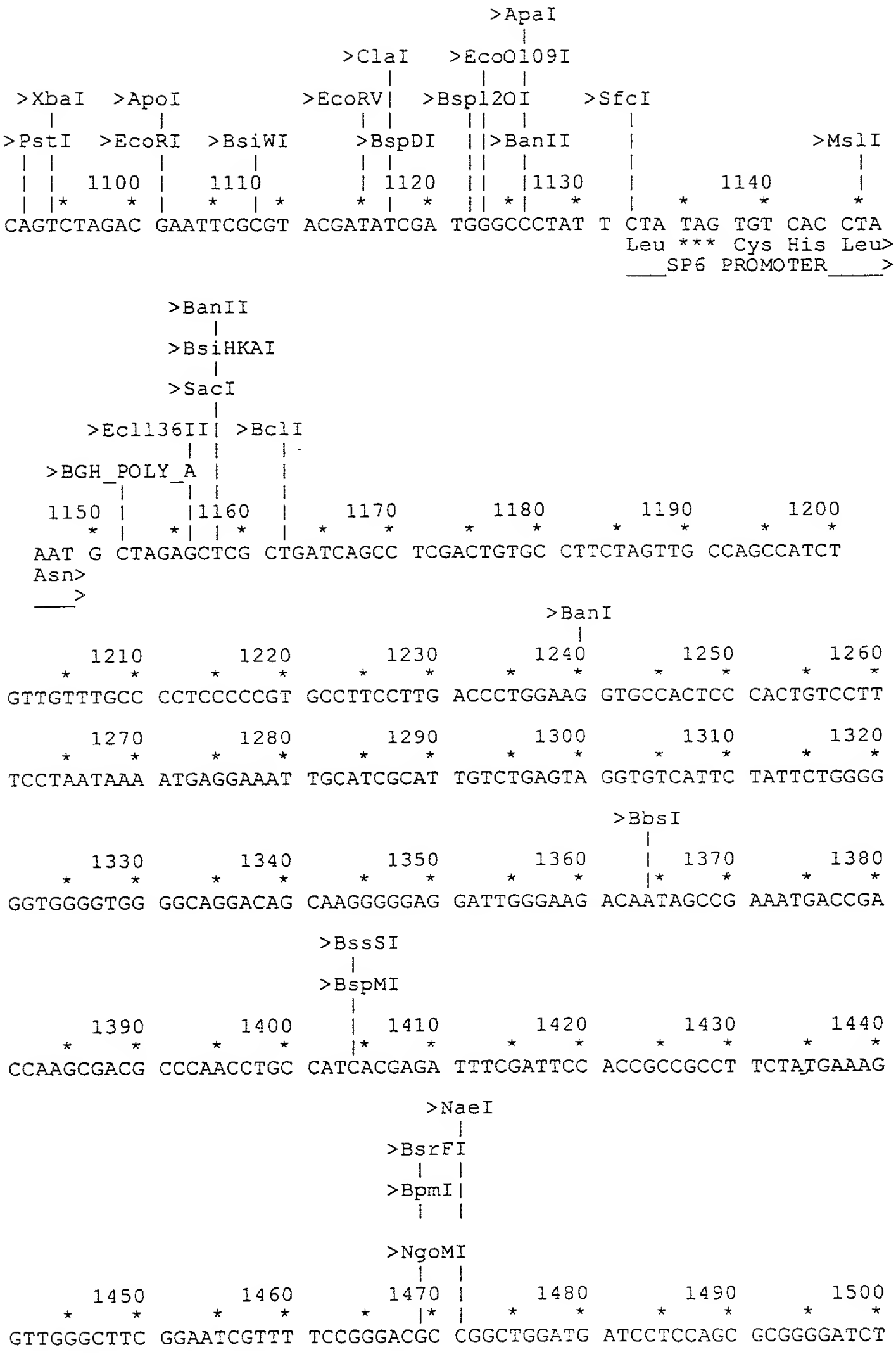


FIG. 4
(CONTINUED)

```
                                    >BpmI
                                      |
                                >SV40_early_poly_A
                                      |
      1510        1520        1530  |     1540        1550        1560
    *    *      *    *      *    *  |  *    *      *    *      *    *
CATGCTGGAG TTCTTCGCCC ACCCCAACTT GTTTATTGCA GCTTATAATG GTTACAAATA

                >ApoI                                 >BsmI
                  |                                     |
      1570        |1580         1590        1600        |1610        1620
    *    *      *  |  *      *    *      *    *      *    |  *      *    *
AAGCAATAGC ATCACAAATT TCACAAATAA AGCATTTTTT TCACTGCATT CTAGTTGTGG

                                                  >HincII
                                                    |
                                        >Bst1107I   >AccI
                                            |       ||
                                       >AccI    >SalI
                                         ||       |||
      1630        1640        1650        1660   ||| 1670        1680
    *    *      *    *      *    *      *  ||  *   ||*    *      *    *
TTTGTCCAAA CTCATCAATG TATCTTATCA TGTCTGTATA CCGTCGACCT CTAGCTAGAG
                                                                    __>
                                                  >      >BsrBI
                                                           |
      1690        1700        1710        1720        1730        1740
    *    *      *    *      *    *      *    *      *    |*      *    *
CTTGGCGTAA TCATGGTCAT AGCTGTTTCC TGTGTGAAAT TGTTATCCGC TCACAATTCC
__________c_________PUC19 BACKBONE H3 TO AATII_________c__________>

                                                        >BanI
                                                          |
          1750        1760        1770        1780      |    1790        1800
        *    *      *    *      *    *      *    *      |  *    *      *    *
      ACACAACATA CGAGCCGGAA GCATAAAGTG TAAAGCCTGG GGTGCCTAAT GAGTGAGCTA
      __________c_________PUC19 BACKBONE H3 TO AATII_________c__________>

           >AseI
             |
          1810        1820        1830        1840        1850        1860
        *  | *      *    *      *    *      *    *      *    *      *    *
      ACTCACATTA ATTGCGTTGC GCTCACTGCC CGCTTTCCAG TCGGGAAACC TGTCGTGCCA
      __________c_________PUC19 BACKBONE H3 TO AATII_________c__________>

  >PvuII
    |
>MspAlI  >AseI      >EaeI                                          >HaeII
    |      |          |                                             |
    |      1870       |1880         1890        1900        1910    | 1920
    |    * | *      *  |  *      *    *      *    *      *    *      *    *
    GCTGCATTAA TGAATCGGCC AACGCGCGGG GAGAGGCGGT TTGCGTATTG GGCGCTCTTC
    __________c_________PUC19 BACKBONE H3 TO AATII_________c__________>

   >EarI
     |
   >SapI                                  >BsiEI              >BsrBI
     |                                      |                   |
     |      1930        1940        1950  |     1960        1970        1980
     |    *    *      *    *      *    *  |  *    *      *    *      *    *
     CGCTTCCTCG CTCACTGACT CGCTGCGCTC GGTCGTTCGG CTGCGGCGAG CGGTATCAGC
     __________c_________PUC19 BACKBONE H3 TO AATII_________c__________>
```

FIG. 4
(CONTINUED)

```
                                                                    >AflIII
                                                                       |
      1990        2000        2010        2020        2030        2040
    *    *      *    *      *    *      *    *      *    *      *  |  *
TCACTCAAAG GCGGTAATAC GGTTATCCAC AGAATCAGGG GATAACGCAG GAAAGAACAT
__________c________PUC19 BACKBONE H3 TO AATII_________c__________>

      2050        2060        2070        2080        2090        2100
    *    *      *    *      *    *      *    *      *    *      *    *
GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT
__________c________PUC19 BACKBONE H3 TO AATII_________c__________>

                                                    >DrdI
                                                      |
      2110        2120        2130        2140      | 2150        2160
    *    *      *    *      *    *      *    *      *    *      *    *
CCATAGGCTC CGCCCCCCTG ACGAGCATCA CAAAAATCGA CGCTCAAGTC AGAGGTGGCG
__________c________PUC19 BACKBONE H3 TO AATII_________c__________>

                                                          >BssSI
                                                            |
      2170        2180        2190        2200        2210        2220
    *    *      *    *      *    *      *    *      *    *      *    *
AAACCCGACA GGACTATAAA GATACCAGGC GTTTCCCCCT GGAAGCTCCC TCGTGCGCTC
__________c________PUC19 BACKBONE H3 TO AATII_________c__________>

                          >BsaWI
                            |
      2230        2240      |  2250       2260        2270        2280
    *    *      *    *      | *    *      *    *      *    *      *    *
TCCTGTTCCG ACCCTGCCGC TTACCGGATA CCTGTCCGCC TTTCTCCCTT CGGGAAGCGT
__________c________PUC19 BACKBONE H3 TO AATII_________c__________>

  >HaeII                >SfcI
    |                     |
    | 2290        2300    |    2310       2320        2330        2340
    *    *      *    *     |  *    *      *    *      *    *      *    *
GGCGCTTTCT CAATGCTCAC GCTGTAGGTA TCTCAGTTCG GTGTAGGTCG TTCGCTCCAA
__________c________PUC19 BACKBONE H3 TO AATII_________c__________>

              >BsiHKAI                    >MspAlI
                 |                          |
          >ApaLI |                    >BsiEI        >BsaWI
             |   |                      | |           |
      2350   |   | 2360       2370      2380        2390        2400
    *    *   |   *    *      *    *     * | |*     *    *      *    *
GCTGGGCTGT GTGCACGAAC CCCCCGTTCA GCCCGACCGC TGCGCCTTAT CCGGTAACTA
__________c________PUC19 BACKBONE H3 TO AATII_________c__________>

                                                            >AlwNI
                                                              |
      2410        2420        2430        2440        2450  |  2460
    *    *      *    *      *    *      *    *      *    *   | *    *
TCGTCTTGAG TCCAACCCGG TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA
__________c________PUC19 BACKBONE H3 TO AATII_________c__________>

                                      >SfcI
                                        |
      2470        2480        2490    |   2500        2510        2520
    *    *      *    *      *    *     | *    *      *    *      *    *
CAGGATTAGC AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA
__________c________PUC19 BACKBONE H3 TO AATII_________c__________>
```

FIG. 4
(CONTINUED)

```
      2530        2540        2550        2560        2570        2580
    *    *      *    *      *    *      *    *      *    *      *    *
CTACGGCTAC ACTAGAAGGA CAGTATTTGG TATCTGCGCT CTGCTGAAGC CAGTTACCTT
___________c________PUC19 BACKBONE H3 TO AATII_________c__________>

 >Eco57I                                          >MspA1I
    |                                                |
    | 2590        2600        2610        2620     | 2630        2640
    *    *      *    *      *    *      *    *     |*    *      *    *
CGGAAAAAGA GTTGGTAGCT CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTTT
___________c________PUC19 BACKBONE H3 TO AATII_________c__________>

      2650        2660        2670        2680        2690        2700
    *    *      *    *      *    *      *    *      *    *      *    *
TTTTGTTTGC AAGCAGCAGA TTACGCGCAG AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT
___________c________PUC19 BACKBONE H3 TO AATII_________c__________>

                                                                >BspHI
                                                                   |
      2710        2720        2730        2740        2750        2760
    *    *      *    *      *    *      *    *      *    *      * |  *
CTTTTCTACG GGGTCTGACG CTCAGTGGAA CGAAAACTCA CGTTAAGGGA TTTTGGTCAT
___________c________PUC19 BACKBONE H3 TO AATII_________c__________>

                                        >DraI                  >DraI
                                          |                      |
      2770        2780        2790      |2800        2810      | 2820
    *    *      *    *      *    *      *|   *      *    *      *    *
GAGATTATCA AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA GTTTTAAATC
___________c________PUC19 BACKBONE H3 TO AATII_________c__________>

                                                                >BanI
                                                                   |
      2830        2840        2850        2860        2870        2880
    *    *      *    *      *    *      *    *      *    *      * |  *
AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA TCAGTGAGGC
                                              ____a____AMP-ORF__________>
___________c________PUC19 BACKBONE H3 TO AATII_________c__________>

                                                          >AhdI
                                                            |
      2890        2900        2910        2920        2930        2940
    *    *      *    *      *    *      *    *      *    *      *    *
ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT GCCTGACTCC CCGTCGTGTA
___________a__________a_____AMP-ORF________a__________a__________>
___________c________PUC19 BACKBONE H3 TO AATII_________c__________>

                                                          >BsaI
                                                            |
                                                          >BsrDI   >BpmI
                                                            |        |
      2950        2960        2970        2980        2990 |      3000
    *    *      *    *      *    *      *    *      *    * |  *   *    *
GATAACTACG ATACGGGAGG GCTTACCATC TGGCCCCAGT GCTGCAATGA TACCGCGAGA
___________a__________a_____AMP-ORF________a__________a__________>
___________c________PUC19 BACKBONE H3 TO AATII_________c__________>

        >BsrFI                                          >BGlI
          |                                               |
      3010        3020        3030        3040        3050        3060
    *    *      *    *      *    *      *    *      *    *      *    *
CCCACGCTCA CCGGCTCCAG ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG
___________a__________a_____AMP-ORF________a__________a__________>
___________c________PUC19 BACKBONE H3 To AATII_________c__________>
```

FIG. 4
(CONTINUED)

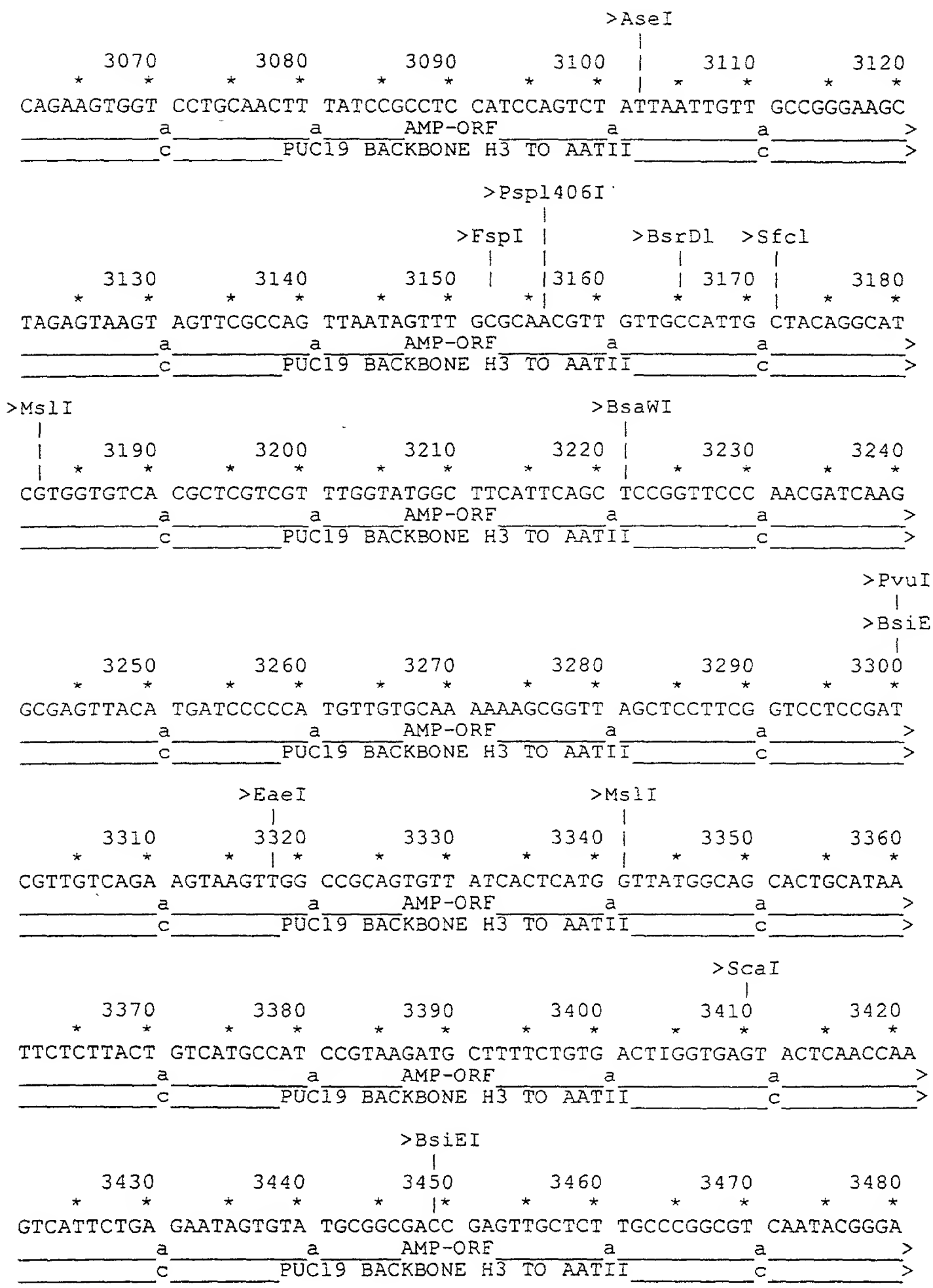


FIG. 4
(CONTINUED)

```
                                                                  >XmnI
                                                                    |
                                                >DraI    >BsiHKAI     >Psp1406I
                                                  |         |          |
        3490        3500        3510        |3520        3530        3540
     *     *     *     *     *  |  *     *  |  *     *     |*     *     *
TAATACCGCG CCACATAGCA GAACTTTAAA AGTGCTCATC ATTGGAAAAC GTTCTTCGGG
__________a__________a_____AMP-ORF_______a__________a__________>
__________c_________PUC19 BACKBONE H3 TO AATII_____c__________>

                                                                  >Eco571
                                                                    |
                                                                  >ApaLI
                                                                    |
                                >MspAlI                           >BssSI
                                   |                              |  |
        3550        3560        | 3570        3580        3590  |  3600
     *     *     *     *     *     *     *     *     *     *     |* |  *
GCGAAAACTC TCAAGGATCT TACCGCTGTT GAGATCCAGT TCGATGTAAC CCACTCGTGC
__________a__________a_____AMP-ORF_______a__________a__________>
__________c_________PUC19 BACKBONE H3 TO AATII_____c__________>

>BsiHKAI
   |
   |    3610        3620        3630        3640        3650        3660
   |  *     *     *     *     *     *     *     *     *     *     *     *
ACCCAACTGA TCTTCAGCAT CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG
__________a__________a_____AMP-ORF_______a__________a__________>
__________c_________PUC19 BACKBONE H3 To AATII_____c__________>

                                                      >MslI
                                                        |
      3670        3680        3690        3700        3710        3720
   *     *     *     *     *     *     *     *     *     *     *     *
AAGGCAAAAT GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT
__________a__________a_____AMP-ORF_____ ___a__________a_______>
__________c_________PUC19 BACKBONE H3 TO AATII_____c__________>

  >EarI     >SspI                                   >BspHI   >BsrBI
    |         |                                       |        |
    | 3730    |  3740       3750        3760        | 3770 |      3780
   *     *    |*     *     *     *     *     *     *     * |  *     *
CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT
__________c_________PUC19 BACKBONE H3 TO AATII_____c__________>

      3790        3800        3810        3820        3830        3840
   *     *     *     *     *     *     *     *     *     *     *     *
ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTC CCCGAAAAGT
__________c_________PUC19 BACKBONE H3 TO AATII_____c__________>

          >HincII
             |
          >AccI
            ||
          >AatII
            ||
        >SalI
          |||
     3850 |||
   *     * |||
GCCACCTGAC GTC
__________c___>
```

FIG. 4
(CONTINUED)

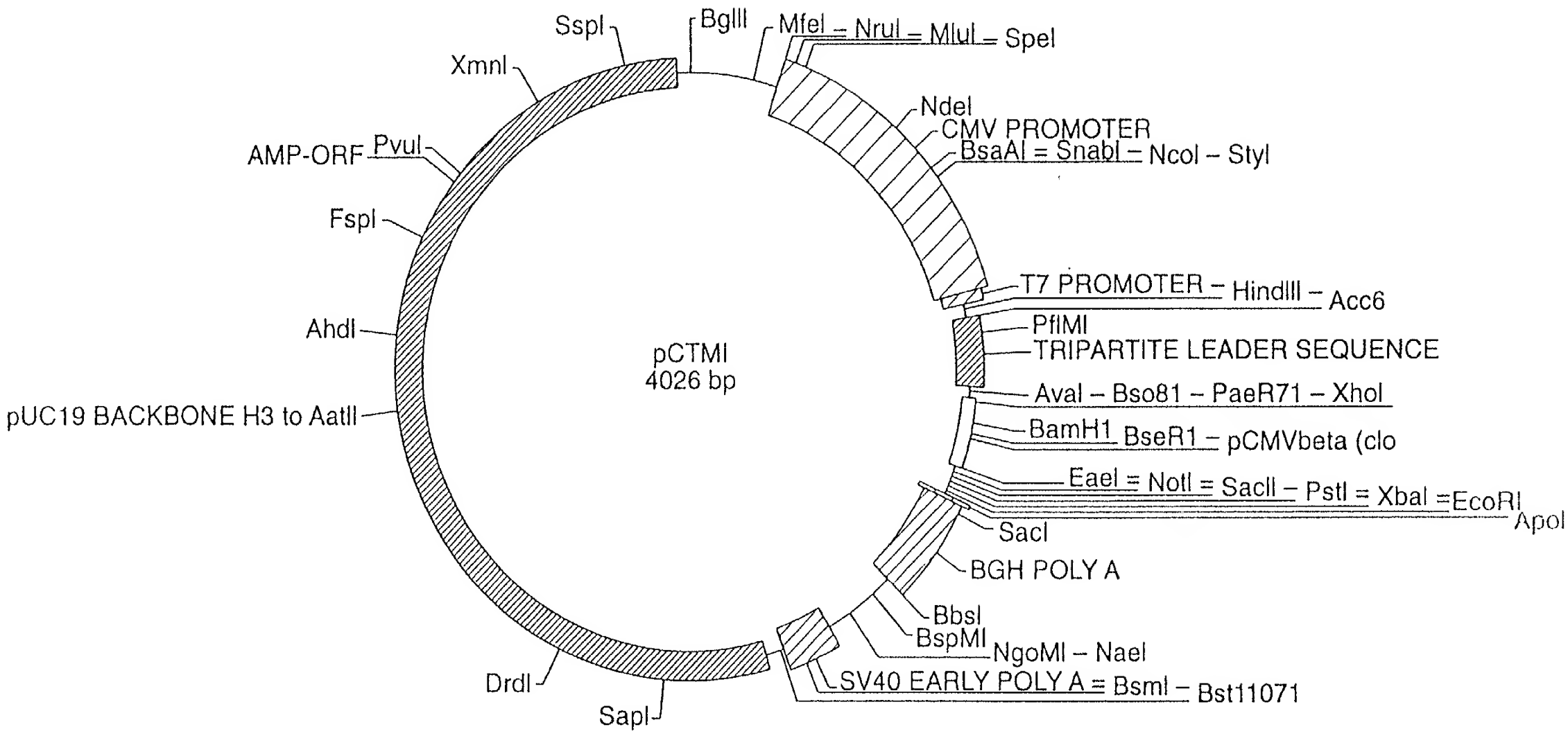

SspI
BglII
MfeI – NruI – MluI – SpeI
XmnI
NdeI
CMV PROMOTER
AMP-ORF
PvuI
BsaAI = SnabI – NcoI – StyI
FspI
T7 PROMOTER – HindIII – Acc6
PflMI
AhdI
pCTMI
4026 bp
TRIPARTITE LEADER SEQUENCE
AvaI – Bso81 – PaeR71 – XhoI
pUC19 BACKBONE H3 to AatII
BamH1 BseR1 – pCMVbeta (clo
EaeI = NotI = SacII – PstI = XbaI = EcoRI
ApoI
SacI
BGH POLY A
BbsI
BspMI
NgoMI – NaeI
DrdI
SV40 EARLY POLY A = BsmI – Bst11071
SapI

FIG. 5

```
                                                    >HincII
                                                       |
                                                    >AccI
                                                     ||
                          >BglII                  >SalI
                             |                      |||
                          10 |        20         30 |||     40          50          60
                      *    * |    *    *       *    * |||*    *       *   *       *   *
GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG

                                >AlwNI
                                   |
                          70          80          90          100         110         120
                      *    *      *    *      *    *      *    *      *    *      *    *
CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG

                      >ApoI                                    >MfeI
                         |                                       |
                         |130          140         150         160 |         170         180
                      *  | *      *    *      *    *      *    * |  *      *   *       *   *
CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC

                                                                                >HincII
                                                                                   |
                                                                 >AflIII           |
                                                                    |              |
                                                  >NruI          >MluI            |
                                                    |              |              |
                          190         200         | 210         220          |230          |
                      *    *      *    *      *   |  *      *    *      *    |  *      |*
TTAGGGTTAG GCGTTTTGCG CTGCTTCG CGA TGT ACG GGC CAG ATA TAC GCG TTG
                                                  Arg Cys Thr Gly Gln Ile Tyr Ala Leu>
                                                  ___e___e___CMV PROMOTER____e___e___>

                                >SpeI       >AseI
                                   |           |
                          240          250     | 260          270          280
                             *       *    |*       *    |   *      *       *       *
ACA TTG ATT ATT GAC TAG TTA TTA ATA GTA ATC AAT TAC GGG GTC ATT
Thr Leu Ile Ile Asp *** Leu Leu Ile Val Ile Asn Tyr Gly Val Ile>
___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>

                               290          300          310          320          330
                           *      *     *      *     *      *     *      *     *      *
AGT TCA TAG CCC ATA TAT GGA GTT CCG CGT TAC ATA ACT TAC GGT AAA
Ser Ser *** Pro Ile Tyr Gly Val Pro Arg Tyr Ile Thr Tyr Gly Lys>
___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>

                                >BglI                                        >AatII
                                   |                                            |
                                340          350          360          370      |
                             *      *     *      *     *      *     *      *     *
TGG CCC GCC TGG CTG ACC GCC CAA CGA CCC CCG CCC ATT GAC GTC AAT
Trp Pro Ala Trp Leu Thr Ala Gln Arg Pro Pro Pro Ile Asp Val Asn>
___e___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>

380          390          400          410          420
  *      *     *      *     *      *     *      *     *      *
AAT GAC GTA TGT TCC CAT AGT AAC GCC AAT AGG GAC TTT CCA TTG ACG
Asn Asp Val Cys Ser His Ser Asn Ala Asn Arg Asp Phe Pro Leu Thr>
___e___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>
```

FIG. 6

```
>AatII                                                  >BglI
 |                                                        |
430                440              450              460  |        470
 | *        *        *        *        *        *         |  *        *        *
TCA ATG GGT GGA CTA TTT ACG GTA AAC TGC CCA CTT GGC AGT ACA TCA
Ser Met Gly Gly Leu Phe Thr Val Asn Cys Pro Leu Gly Ser Thr Ser>
___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>

          >NdeI                                          >AatII
            |                                               |
   480      |      490             500             510|         520
    *       | *      *       *        *       *       *|     *       *
AGT GTA TCA TAT GCC AAG TAC GCC CCC TAT TGA CGT CAA TGA CGG TAA
Ser Val Ser Tyr Ala Lys Tyr Ala Pro Tyr *** Arg Gln *** Arg ***>
___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>

             >BglI
               |
       530     |      540            550            560           570
 *       *     | *      *       *       *       *       *      *       *
ATG GCC CGC CTG GCA TTA TGC CCA GTA CAT GAC CTT ATG GGA CTT TCC
Met Ala Arg Leu Ala Leu Cys Pro Val His Asp Leu Met Gly Leu Ser>
___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>

                          >BsaAI                         >NcoI
                            |                              |
                          >SnaBI                         >StyI   >MslI
                            |                              |        |
          580              590            600            610        |
      *       *       *       *       *       *       *       *       *
TAC TTG GCA GTA CAT CTA CGT ATT AGT CAT CGC TAT TAC CAT GGT GAT
Tyr Leu Ala Val His Leu Arg Ile Ser His Arg Tyr Tyr His Gly Asp>
___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>

620            630             640            650            660
 *      *       *       *      *       *       *      *       *       *
GCG GTT TTG GCA GTA CAT CAA TGG GCG TGG ATA GCG GTT TGA CTC ACG
Ala Val Leu Ala Val His Gln Trp Ala Trp Ile Ala Val *** Leu Thr>
___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>

                                             >AatII                 >BanI
                                               |                      |
670              680             690           | 700          710     |
 *        *        *        *       *       *  |  *       *       *   *
GGG ATT TCC AAG TCT CCA CCC CAT TGA CGT CAA TGG GAG TTT GTT TTG
Gly Ile Ser Lys Ser Pro Pro His *** Arg Gln Trp Glu Phe Val Leu>
___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>

   720           730             740            750            760
    *       *      *       *       *       *      *       *      *
GCA CCA AAA TCA ACG GGA CTT TCC AAA ATG TCG TAA CAA CTC CGC CCC
Ala Pro Lys Ser Thr Gly Leu Ser Lys Met Ser *** Gln Leu Arg Pro>
___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>

       770            780             790            800            810
 *       *      *       *       *      *       *       *      *       *
ATT GAC GCA AAT GGG CGG TAG GCG TGT ACG GTG GGA GGT CTA TAT AAG
Ile Asp Ala Asn Gly Arg *** Ala Cys Thr Val Gly Gly Leu Tyr Lys>
___e___e___e___e___e___e_CMV PROMOTER__e___e___e___e___e___e___>
```

FIG. 6
(CONTINUED)

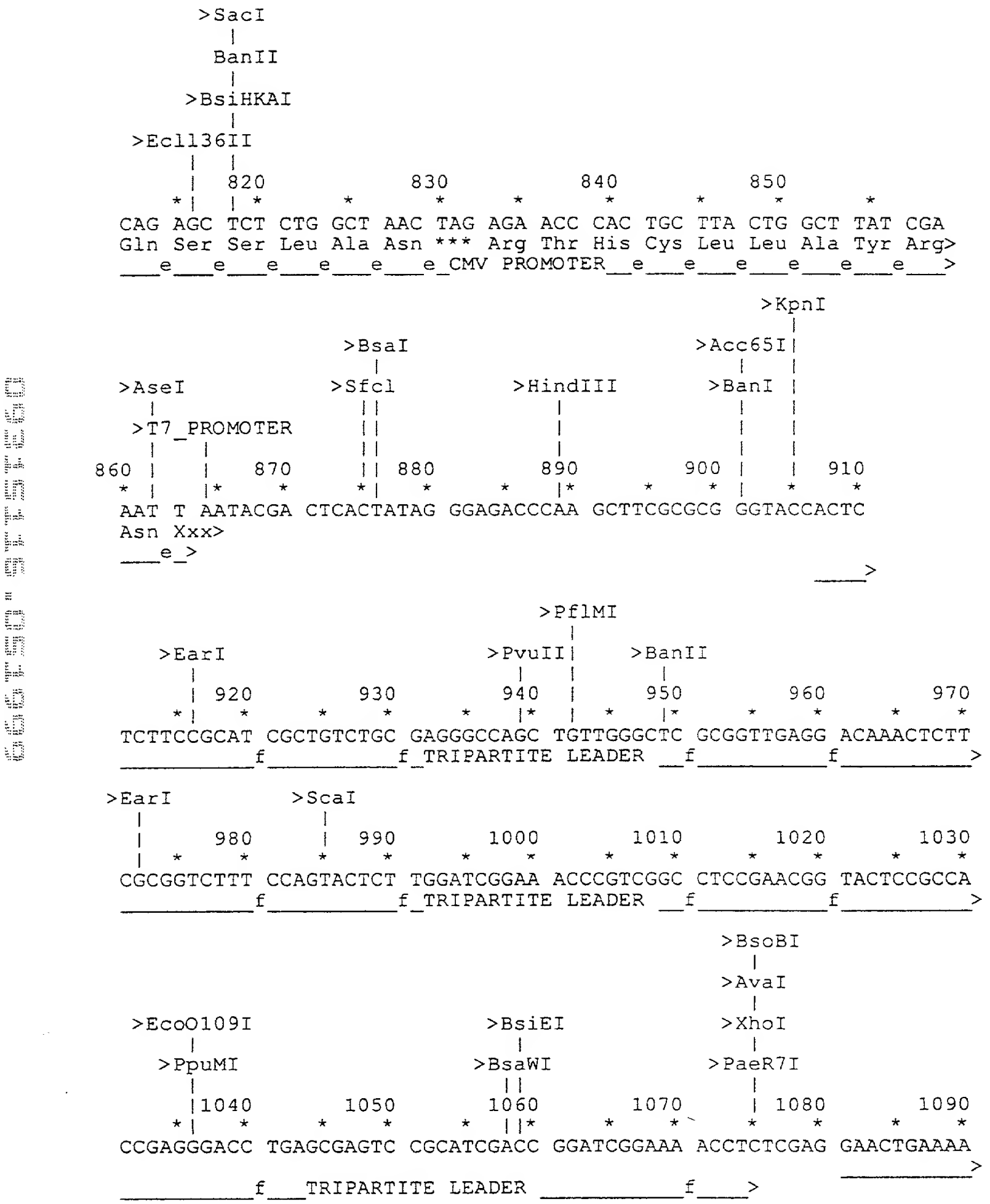


FIG. 6
(CONTINUED)

```
                >HincII                                        >Eco0109I   >BsaWI
                  |                                                |             |
                 >HpaI                                         >PpuMI    >BamHI
                  |                                                |          |  |
       1100       |    1110         1120         1130         1140         |  1150
          *       *  |    *        *        *        *        *        *        *   |  *  |*  |  *
ACCAGAAAGT TAACTGGTAA GTTTAGTCTT TTTGTCTTTT TATTTCAGGT CCCGGATCCG
__________b__________ HYBRID SV40 LATE INTRON__________b__________>

                 >BseRI                                          >StuI
                    |                                               |
       1160         |1170         1180         1190         1200      | 1210
          *        *        *|        *        *        *        *        *        *        *
GTGGTGGTGC AAATCAAAGA ACTGCTCCTC AGTGGATGTT GCCTTTACTT CTAGGCCTGT
__________b__________ HYBRID SV40 LATE INTRON__________b__________>

                                                            >BsiEI
                                                               |
                                                        >EagI|
                                                            |  |
                                                        >EaeI|        >XbaI
                                                            |  |             |
                                                        >SacII       >PstI
                                                            |  |          |  |
                                                      >NotI | >SfcI  |  |
                                                            |  |      |    |  |
       1220         1230         1240         1250         | 1260 |    | 1270
          *        *        *        *        *        *        *        *        *   |  *   |  *  |  *
ACGGAAGTGT TACTTCTGCT CTAAAAGCTG CGGAATTGTA CCCGCGGCCG CTGCAGTCTA
____________HYBRID SV40 LATE INTRON________b___>

                                          >ApaI
                                             |
                     >BspDI     >Eco0109I
                          |              |  |
  >ApoI          >EcoRV|   >Bsp120I
     |                 |  |          ||  |
>EcoRI    >BsiWI     >ClaI      |>BanII      >SfcI            >MslI
     |          |          |  |          ||  |          |                     |
     |  1280    |     1290     ||1300        |    1310           1320
     |*        *   |    *   |  *        *|    |*        *        *        *       |  *
GACGAATTCG CGTACGATAT CGATGGGCCC TATT CTA TAG TGT CAC CTA AAT
                                                   Leu *** Cys His Leu Asn>
                                                   ___c_SP6 PROMOTER__c___>

        >SacI
           |
        >BanII
           |
       >BsiHKAI
           |
  >Ecl136II | >BclI
         |   |     |
>BGH_POLY_A  |     |
    |    |   |     |
    |  1330  |     | 1340       1350         1360         1370         1380
    |*   |*  |     *        *        *        *        *        *        *        *        *
GCTAGAGC TCGCTGATCA GCCTCGACTG TGCCTTCTAG TTGCCAGCCA TCTGTTGTTT

                                            >BanI
                                               |
       1390         1400         1410      |  1420         1430         1440
          *        *        *        *        *        *   |  *        *        *        *        *
GCCCCTCCCC CGTGCCTTCC TTGACCCTGG AAGGTGCCAC TCCCACTGTC CTTTCCTAAT
```

FIG. 6
(CONTINUED)

```
      1450        1460        1470        1480        1490        1500
    *     *     *     *     *     *     *     *     *     *     *     *
AAAATGAGGA AATTGCATCG CATTGTCTGA GTAGGTGTCA TTCTATTCTG GGGGGTGGGG

                                          >BbsI
                                            |
      1510        1520        1530        1540        1550        1560
    *     *     *     *     *     *     *   |  *     *     *     *     *
TGGGGCAGGA CAGCAAGGGG GAGGATTGGG AAGACAATAG CCGAAATGAC CGACCAAGCG

                  >BspMI
                    |
                  >BssSI
                    |
      1570        1580        1590        1600        1610        1620
    *     *     *   |  *     *     *     *     *     *     *     *     *
ACGCCCAACC TGCCATCACG AGATTTCGAT TCCACCGCCG CCTTCTATGA AAGGTTGGGC

                          >NaeI
                            |
                        >NgoMI
                          | |
                        >BpmI
                          | |
                        >BsrFI
                          | |
      1630        1640    | |  1650       1660        1670        1680
    *     *     *     *     | |  *     *     *     *     *     *     *     *
TTCGGAATCG TTTTCCGGGA CGCCGGCTGG ATGATCCTCC AGCGCCGGGA TCTCATGCTG

                        >BpmI
                          |
                  >SV40_early_poly_A
                          |
      1690        1700        1710        1720        1730        1740
    *     *     *     |*     *     *     *     *     *     *     *     *
GAGTTCTTCG CCCACCCCAA CTTGTTTATT GCAGCTTATA ATGGTTACAA ATAAAGCAAT

        >ApoI                                 >BsmI
          |                                     |
      1750        1760        1770        1780        1790        1800
    *     |*    *     *     *     *     *     |*    *     *     *     *     *
AGCATCACAA ATTTCACAAA TAAAGCATTT TTTTCACTGC ATTCTAGTTG TGGTTTGTCC

                                          >HincII
                                            |
                                  >BstI107I   >AccI
                                      |       ||
                                    >AccI|  >SalI
                                       | |    |||
      1810        1820        1830     |    |1840        1850        1860
    *     *     *     *     *     *  |  *|||  *     *     *     *     *     *
AAACTCATCA ATGTATCTTA TCATGTCTGT ATACCGTCGA CCTCTAGCTA GAGCTTGGCG
                                                          _________>

                                                  >BsrBI
                                                    |
      1870        1880        1890        1900    |   1910        1920
    *     *     *     *     *     *     *     *     |*    *     *     *     *     *
TAATCATGGT CATAGCTGTT TCCTGTGTGA AATTGTTATC CGCTCACAAT TCCACACAAC
__________d__________d___PUC19 BACKBONE____d__________d__________>
```

FIG. 6
(CONTINUED)

```
                                                  >BanI
                                                    |
      1930        1940        1950        1960    |   1970        1980
    *    *      *    *      *    *      *    *     |*    *      *    *
ATACGAGCCG GAAGCATAAA GTGTAAAGCC TGGGGTGCCT AATGAGTGAG CTAACTCACA
__________d__________d___PUC19 BACKBONE____d__________d__________>

                                                                    >AseI
                                                                      |
>AseI                                                    >PvuII       |
  |                                                        |          |
  |     1990        2000        2010        2020        2030    |  2040
  |  *    *      *    *      *    *      *    *      *    *     |* |  *
  TTAATTGCGT TGCGCTCACT GCCCGCTTTC CAGTCGGGAA ACCTGTCGTG CCAGCTGCAT
  __________d__________d___PUC19 BACKBONE____d__________d__________>

                                                          >EarI
                                                            |
      >EaeI                                       >HaeII    >SapI
        |                                           |         |
      2050        2060        2070        2080        2090    |  2100
    *   |*     *    *      *    *      *    *      *   | *    |* |  *
  TAATGAATCG GCCAACGCGC GGGGAGAGGC GGTTTGCGTA TTGGGCGCTC TTCCGCTTCC
  __________d__________d___PUC19 BACKBONE____d__________d__________>

                              >BsiEI                >BsrBI
                                |                     |
      2110        2120        |2130        2140     |   2150        2160
    *    *      *    *      *  |  *      *    *     | *    *      *    *
  TCGCTCACTG ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA
  __________d__________d___PUC19 BACKBONE____d__________d__________>

                                                        >AflIII
                                                          |
      2170        2180        2190        2200        2210        2220
    *    *      *    *      *    *      *    *      *    *      *    *
  AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG CAGGAAAGAA CATGTGAGCA
  __________d__________d___PUC19 BACKBONE____d__________d__________>

      2230        2240        2250        2260        2270        2280
    *    *      *    *      *    *      *    *      *    *      *    *
  AAAGGCCAGC AAAAGGCCAG GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG
  __________d__________d___PUC19 BACKBONE____d__________d__________>

                                          >DrdI
                                            |
      2290        2300        2310        2320        2330        2340
    *    *      *    *      *    *      *   | *    *      *    *      *
  CTCCGCCCCC CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG
  __________d__________d___PUC19 BACKBONE____d__________d__________>

                                                  >BssSI
                                                    |
      2350        2360        2370        2380    |   2390        2400
    *    *      *    *      *    *      *    *     | *    *      *    *
  ACAGGACTAT AAAGATACCA GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT

  __________d__________d___PUC19 BACKBONE____d__________d__________>
```

FIG. 6
(CONTINUED)

```
                >BsaWI                                                  >HaeII
                   |                                                      |
      2410         |2420         2430         2440         2450         2460
    *    *       *    *       *    *       *    *       *    *       *  |  *
CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTCGGGAAG CGTGGCGCTT
__________d__________d___PUC19 BACKBONE____d__________d__________>

               >SfcI
                  |
      2470        | 2480         2490         2500         2510         2520
    *    *      *    *       *    *       *    *       *    *       *    *
TCTCAATGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC
__________d__________d___PUC19 BACKBONE____d__________d__________>

    >BsiHKAI
        |
 >ApaLI|                          >BsiEI          >BsaWI
   |   |                            |               |
   |  2530         2540         2550         2560   |  2570         2580
   |*  | *       *    *       *    *       *    *   | *    *       *    *
TGTGTGCACG AACCCCCCGT TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT
__________d__________d___PUC19 BACKBONE____d__________d__________>

                                                    >AlwNI
                                                       |
      2590         2600         2610         2620      |2630         2640
    *    *       *    *       *    *       *    *      *|   *       *    *
GAGTCCAACC CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT
__________d__________d___PUC19 BACKBONE____d__________d__________>

                              >SfcI
                                 |
      2650         2660         |2670         2680         2690         2700
    *    *       *    *       *  |  *       *    *       *    *       *    *
AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC
__________d__________d___PUC19 BACKBONE____d__________d__________>

                                                                >Eco57I
                                                                   |
      2710         2720         2730         2740         2750    |    2760
    *    *       *    *       *    *       *    *       *    *    |  *
TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTCGGAAAA
__________d__________d___PUC19 BACKBONE____d__________d__________>

      2770         2780         2790         2800         2810         2820
    *    *       *    *       *    *       *    *       *    *       *    *
AGAGTTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG GTAGCGGTGG TTTTTTTGTT
__________d__________d___PUC19 BACKBONE____d__________d__________>

      2830         2840         2850         2860         2870         2880
    *    *       *    *       *    *       *    *       *    *       *    *
TGCAAGCAGC AGATTACGCG CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT
__________d__________d___PUC19 BACKBONE____d__________d__________>

                                                          >BspHI
                                                             |
      2890         2900         2910         2920         2930         2940
    *    *       *    *       *    *       *    *       *    *       *    *
ACGGGGTCTG ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA
__________d__________d___PUC19 BACKBONE____d__________d__________>
```

FIG. 6
(CONTINUED)

```
                                        >DraI                   >DraI
                                         |                       |
      2950        2960        2970        2980        2990        3000
    *   *     *   *   *     *   |*    *   *   *     *   | *   *     *
TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA
__________d__________d___PUC19 BACKBONE____d__________d__________>

                                                              >BanI
                                                               |
      3010        3020        3030        3040        3050   |    3060
    *   *     *   *   *     *   *   *     *   *     *   *   |  *   *
AGTATATATG AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC
                              _a__________AMP-ORF___a__________>
__________d__________d___PUC19 BACKBONE____d__________d__________>

                                                    >AhdI
                                                     |
      3070        3080        3090        3100       |    3110        3120
    *   *     *   *   *     *   *   *     *   *       | *   *     *   *
TCAGCGATCT GTCTATTTCG TTCATCCATA GTTGCCTGAC TCCCCGTCGT GTAGATAACT
__________a__________a_____AMP-ORF_________a__________a__________>
__________d__________d___PUC19 BACKBONE____d__________d__________>

                                                      >BsaI
                                                       |
                                                     >BsrDI     >BpmI
                                                       |          |
      3130        3140        3150        3160       |  3170    |    3180
    *   *     *   *   *     *   *   *     *   *       |* *   *  | *  *
ACGATACGGG AGGGCTTACC ATCTGGCCCC AGTGCTGCAA TGATACCGCG AGACCCACGC
__________a__________a_____AMP-ORF_________a__________a__________>
__________d__________d___PUC19 BACKBONE____d__________d__________>

>BsrFI                                                >BglI
   |                                                    |
   |  3190        3200        3210        3220          |    3230        3240
   | *   *     *   *   *     *   *   *     *   *         | *   *     *   *
TCACCGGCTC CAGATTTATC AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT
__________a__________a_____AMP-ORF_________a__________a__________>
__________d__________d___PUC19 BACKBONE____d__________d__________>

                                          >AseI
                                           |
      3250        3260        3270        | 3280        3290        3300
    *   *     *   *   *     *   *   *     *   *     *   *   *     *   *
GGTCCTGCAA CTTTATCCGC CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA
__________a__________a_____AMP-ORF_________a__________a__________>
__________d__________d___PUC19 BACKBONE____d__________d__________>

                                  >Psp1406I
                                   |
                          >FspI    |     >BsrDI   >SfcI        >MslI
                            |      |       |        |           |
      3310        3320      | 3330       3340     |  3350     | 3360
    *   *     *   *   *     *   |*    *   *   *     *   *     *   *
AGTAGTTCGC CAGTTAATAG TTTGCGCAAC GTTGTTGCCA TTGCTACAGG CATCGTGGTG
__________a__________a_____AMP-ORF_________a__________a__________>
__________d__________d___PUC19 BACKBONE____d__________d__________>
```

FIG. 6
(CONTINUED)

```
                                                                >BsaWI
                                                                  |
      3370        3380        3390        3400        3410        3420
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TCACGCTCGT CGTTTGGTAT GGCTTCATTC AGCTCCGGTT CCCAACGATC AAGGCGAGTT
__________a__________a_____AMP-ORF_____a__________a__________>
__________d__________d___PUC19 BACKBONE___d__________d__________>

                                                                >BsiEI
                                                                  |
                                                                >PvuI
                                                                  |
      3430        3440        3450        3460        3470      |  3480
    *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   | *   *
ACATGATCCC CCATGTTGTG CAAAAAAGCG GTTAGCTCCT TCGGTCCTCC GATCGTTGTC
__________a__________a_____AMP-ORF_____a__________a__________>
__________d__________d___PUC19 BACKBONE___d__________d__________>

          >EaeI                                 >MslI
            |                                     |
      3490  |     3500        3510        3520        3530        3540
    *   *   |   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AGAAGTAAGT TGGCCGCAGT GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT
__________a__________a_____AMP-ORF_____a__________a__________>
__________d__________d___PUC19 BACKBONE___d__________d__________>

                                                        >ScaI
                                                          |
 3500                3560        3570        3580      |  3590        3600
    *   *   *   *   *   *   *   *   *   *   *   *   *   | *   *   *   *
ACTGTCATGC CATCCGTAAG ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC
__________a__________a_____AMP-ORF_____a__________a__________>
__________d__________d___PUC19 BACKBONE___d__________d__________>

                        >BsiEI
                          |
      3610        3620  |     3630        3640        3650        3660
    *   *   *   *   *   | *   *   *   *   *   *   *   *   *   *   *   *
TGAGAATAGT GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG GGATAATACC
__________a__________a_____AMP-ORF_____a__________a__________>
__________d__________d___PUC19 BACKBONE___d__________d__________>

                                                  >Pspl406I
                                                    |
                    >DraI     >BsiHKAI          >XmnI
                      |         |                 |
      3670        3680        3690        3700  |     3710        3720
    *   *   *   *   *   *   *   |*  *   *   *   | *   *   *   *   *   *
GCGCCACATA GCAGAACTTT AAAAGTGCTC ATCATTGGAA AACGTTCTTC GGGGCGAAAA
__________a__________a_____AMP-ORF_____a__________a__________>
__________d__________d___PUC19 BACKBONE___d__________d__________>

                                                      >ApaLI
                                                        |
                                                    >Eco57I
                                                        |
                                                >BssSI  | >BsiHKAI
                                                    |   |     |
      3730        3740        3750        3760        3770    |  3780
    *   *   *   *   *   *   *   *   *   *   *   *   *   | *   |*   *
CTCTCAAGGA TCTTACCGCT GTTGAGATCC AGTTCGATGT AACCCACTCG TGCACCCAAC
__________a__________a_____AMP-ORF_____a__________a__________>
__________d__________d___PUC19 BACKBONE___d__________d__________>
```

FIG. 6
(CONTINUED)

```
      3790        3800        3810        3820        3830        3840
    *    *      *    *      *    *      *    *      *    *      *    *
TGATCTTCAG CATCTTTTAC TTTCACCAGC GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA
__________a__________a_____AMP-ORF_________a__________a__________>
__________d__________d___PUC19 BACKBONE____d__________d__________>

                                    >MsiI                     >EarI
                                      |                         |
      3850        3860        3870    |   3880        3890      3900
    *    *      *    *      *    *    | *    *      *    *    *  | *
AATGCCGCAA AAAAGGGAAT AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT
__________a__________a_____AMP-ORF_________a__________>
__________d__________d___PUC19 BACKBONE____d__________d__________>

    >SspI                               >BspHI   >BsrBI
      |                                   |        |
      3910        3920        3930        3940     |  3950        3960
    *  |  *      *    *      *    *      *  |  *     |* *    *      *    *
TTTCAATATT ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA
__________d__________d___PUC19 BACKBONE____d__________d__________>

      3970        3980        3990        4000        4010        4020
    *    *      *    *      *    *      *    *      *    *      *    *
TGTATTTAGA AAAATAAACA ATAGGGGTT CCGCGCACAT TTCCCCGAAA AGTGCCACCT
__________d__________d___PUC19 BACKBONE____d__________d__________>

  >HincII
     |
  >AatII
    ||
  >AccI
    ||
 >SalI
   |||
   |*|
GACGTC
______>
```

FIG. 6
(CONTINUED)

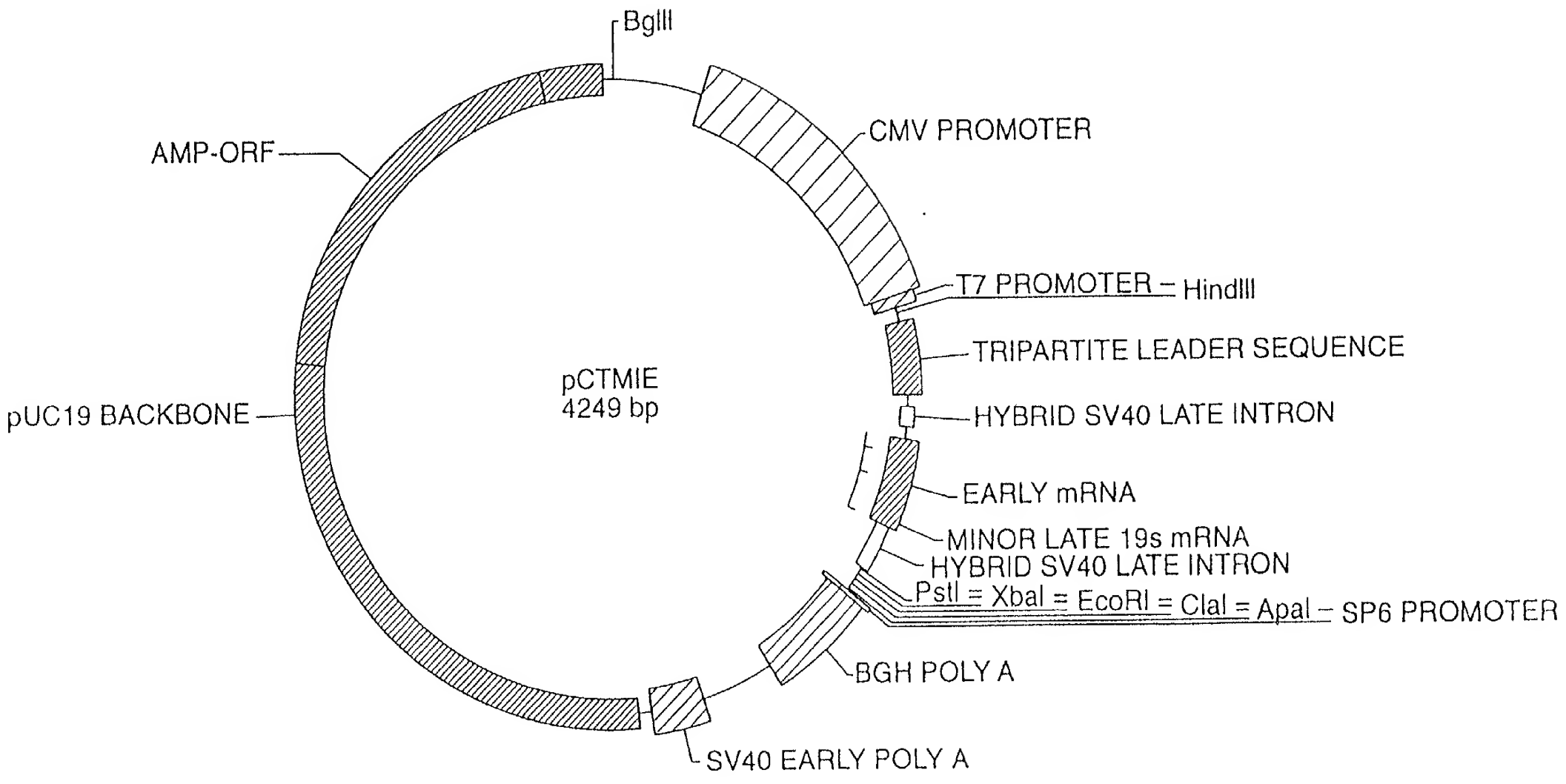

BglII
AMP-ORF
CMV PROMOTER
T7 PROMOTER – HindIII
TRIPARTITE LEADER SEQUENCE
pCTMIE
4249 bp
pUC19 BACKBONE
HYBRID SV40 LATE INTRON
EARLY mRNA
MINOR LATE 19s mRNA
HYBRID SV40 LATE INTRON
PstI = XbaI = EcoRI = ClaI = ApaI – SP6 PROMOTER
BGH POLY A
SV40 EARLY POLY A

FIG. 7

```
                                                    >HincII
                                                      |
                                                    >AccI
                                                     ||
                                >BglII              >SalI
                                  |                 |||
                          10      |       20      30|||     40        50        60
                      *     *     |   *     *     *   *|||*     *     *     *     *     *
GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG

                                >AlwNI
                                  |
                          70        80        90        100       110       120
                      *     *     *   *     *   *     *   *     *   *     *   *     *
CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG

                                                    >MfeI
                                                      |
    >ApoI                                             |
      |                                               |
      |130        140       150       160           |       170       180
    * | *     *   *     *   *     *   *     *         | *   *     *   *     *
CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC

                                                                        >HincII
                                                                          |
                                                                          |
                                                          >AflIII         |
                                                            |             |
                                                          >MluI           |
                                                            |             |
                                  >NruI                     |             |
                                    |                       |             |
                          190       |200        220         |230          |
                      *     *     *   |*     *     *     *   |   *     *   |*
TTAGGGTTAG GCGTTTTGCG CTGCTTCG CGA TGT ACG GGC CAG ATA TAC GCG TTG
                                 Arg Cys Thr Gly Gln Ile Tyr Ala Leu>
                                 ___f___f___CMV PROMOTER____f___f___>

                                >SpeI       >AseI
                                  |           |
                          240     |250        | 260         270         280
                      *     *     |   *     * |   *     *   *     *   *     *
ACA TTG ATT ATT GAC TAG TTA TTA ATA GTA ATC AAT TAC GGG GTC ATT
Thr Leu Ile Ile Asp *** Leu Leu Ile Val Ile Asn Tyr Gly Val Ile>
___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>

                          290       300       310       320       330
                      *     *     *   *     *   *     *   *     *   *
AGT TCA TAG CCC ATA TAT GGA GTT CCG CGT TAC ATA ACT TAC GGT AAA
Ser Ser *** Pro Ile Tyr Gly Val Pro Arg Tyr Ile Thr Tyr Gly Lys>
___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>

                  >BglI                                                 >AatII
                    |                                                     |
                  340       350       360       370                     |
                *     *   *     *   *     *   *     *   *
TGG CCC GCC TGG CTG ACC GCC CAA CGA CCC CCG CCC ATT GAC GTC AAT
Trp Pro Ala Trp Leu Thr Ala Gln Arg Pro Pro Pro Ile Asp Val Asn>
___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>

380       390       400       410       420
  *     *   *     *   *     *   *     *   *     *
AAT GAC GTA TGT TCC CAT AGT AAC GCC AAT AGG GAC TTT CCA TTG ACG
Asn Asp Val Cys Ser His Ser Asn Ala Asn Arg Asp Phe Pro Leu Thr>
___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>
```

FIG. 8

```
>AatII                                                    >BglI
  |                                                         |
  430           440           450           460           |       470
  | *       *       *       *       *       *       *     |   *       *       *
  TCA ATG GGT GGA CTA TTT ACG GTA AAC TGC CCA CTT GGC AGT ACA TCA
  Ser Met Gly Gly Leu Phe Thr Val Asn Cys Pro Leu Gly Ser Thr Ser>
  ___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>

          >NdeI                                           >AatII
            |                                               |
     480    |      490           500           510          |       520
       *    | *     *       *     *       *     *          |   *       *
  AGT GTA TCA TAT GCC AAG TAC GCC CCC TAT TGA CGT CAA TGA CGG TAA
  Ser Val Ser Tyr Ala Lys Tyr Ala Pro Tyr *** Arg Gln *** Arg ***>
  ___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>

            >BglI
              |
        530   |       540           550           560           570
  *       *   | *       *       *     *       *     *       *     *
  ATG GCC CGC CTG GCA TTA TGC CCA GTA CAT GAC CTT ATG GGA CTT TCC
  Met Ala Arg Leu Ala Leu Cys Pro Val His Asp Leu Met Gly Leu Ser>
  ___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>

                          >BsaAI                          >StyI
                            |                               |
                          >SnaBI                          >NcoI    >MslI
                            |                               |       |
            580           590           600           610   |
       *       *       *       *       *       *       *     *
  TAC TTG GCA GTA CAT CTA CGT ATT AGT CAT CGC TAT TAC CAT GGT GAT
  Tyr Leu Ala Val His Leu Arg Ile Ser His Arg Tyr Tyr His Gly Asp>
  ___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>

620           630           640           650           660
  *       *       *       *       *       *       *       *
  GCG GTT TTG GCA GTA CAT CAA TGG GCG TGG ATA GCG GTT TGA CTC ACG
  Ala Val Leu Ala Val His Gln Trp Ala Trp Ile Ala Val *** Leu Thr>
  ___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>

                                                >AatII                  >BanI
                                                  |                       |
  670           680           690               | 700           710     |
    *       *       *       *       *       *   |   *       *       *   |
  GGG ATT TCC AAG TCT CCA CCC CAT TGA CGT CAA TGG GAG TTT GTT TTG
  Gly Ile Ser Lys Ser Pro Pro His *** Arg Gln Trp Glu Phe Val Leu>
  ___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>

     720           730           740           750           760
       *       *       *       *       *       *       *     *
  GCA CCA AAA TCA ACG GGA CTT TCC AAA ATG TCG TAA CAA CTC CGC CCC
  Ala Pro Lys Ser Thr Gly Leu Ser Lys Met Ser *** Gln Leu Arg Pro>
  ___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>

        770           780           790           800           810
  *       *       *       *       *       *       *       *     *
  ATT GAC GCA AAT GGG CGG TAG GCG TGT ACG GTG GGA GGT CTA TAT AAG
  Ile Asp Ala Asn Gly Arg *** Ala Cys Thr Val Gly Gly Leu Tyr Lys>
  ___f___f___f___f___f___f_CMV PROMOTER__f___f___f___f___f___f___>
```

FIG. 8
(CONTINUED)

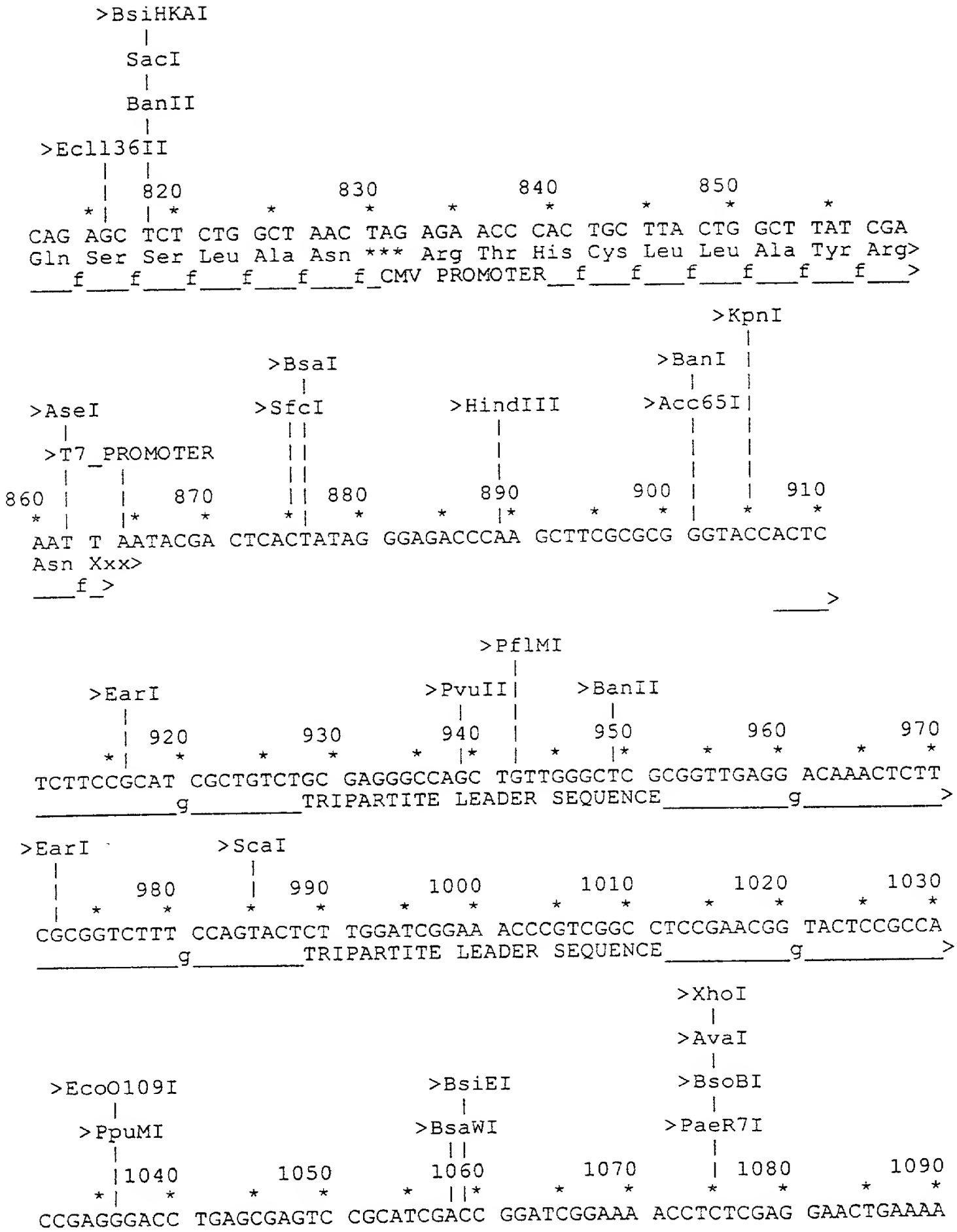


FIG. 8
(CONTINUED)

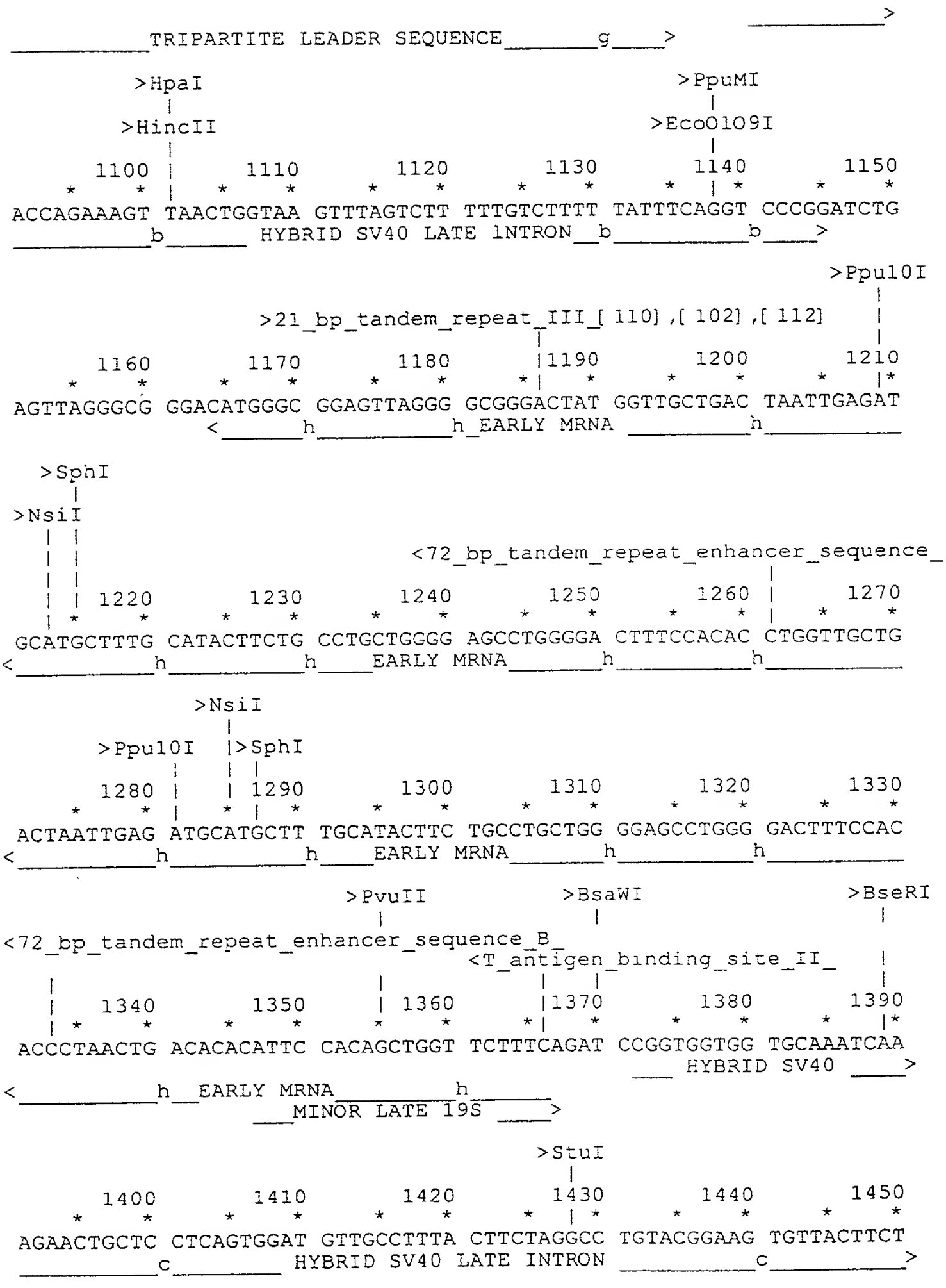


FIG. 8
(CONTINUED)

```
                                      >BsiEI
                                          |
                                  >NotI  |
                                     |   |
                                  >EaeI  |        >XbaI
                                     |   |            |
                                  >SacII|     >PstI      >EcoRI
                                     |   |        | |        |
                                  >EagI |>SfcI | |     >ApoI    >BsiWI
                                     |   |   |    | |        |        |
      1460       1470       1480  |   |  1490       1500     |  1510
         *    *     *    *     *  | * |  |*   | *      *  |  *    |*    *
GCTCTAAAAG CTGCGGAATT GTACCCGCGG CCGCTGCAGT CTAGACGAAT TCGCGTACGA
____ HYBRID SV40 LATE INT____>

              >ApaI                                               >SacI
                 |                                                   |
  >BspDI   >BanII                                            >BsiHKAI
      |          |                                                   |
  >ClaI   >Eco0109I                                            >BanII
      |       |  |                                                   |
>EcoRV|  >Bsp120I    >SfcI             >MslI    >Ecl136II|  >BclI
   |  |      ||  |        |                 |             | |     |
   |  |      ||  |        |                 |  >BGH_POLY_A| |     |
   |  |      ||  |        |                 |        |    | |     |
   |  |    1520  |       1530          1540|     1550   | |   1560
   |  | *   ||*  |   *     | *      *     *|    *   |  *  | |*  | *
TATCGATGGG CCCTATT CTA TAG TGT CAC CTA AAT GCTAG AGCTCGCTGA
                   Leu *** Cys His Leu Asn>
                   ___d_SP6 PROMOTER__d___>

      1570       1580       1590       1600       1610       1620
         *    *     *    *     *    *     *    *     *    *     *
TCAGCCTCGA CTGTGCCTTC TAGTTGCCAG CCATCTGTTG TTTGCCCCTC CCCCGTGCCT

               >BanI
                   |
      1630        |1640       1650       1660       1670       1680
         *    *    *|   *     *    *     *    *     *    *     *
TCCTTGACCC TGGAAGGTGC CACTCCCACT GTCCTTTCCT AATAAAATGA GGAAATTGCA

      1690       1700       1710       1720       1730       1740
         *    *     *    *     *    *     *    *     *    *     *
TCGCATTGTC TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG

                                                              >BspMI
                                                                   |
                    >BbsI                                     >BssSI
                        |                                          |
      1750       1760       1770       1780       1790       1800
         *    *     *    *     *    *     *    *     *    *     *
GGGGAGGATT GGGAAGACAA TAGCCGAAAT GACCGACCAA GCGACGCCCA ACCTGCCATC

      1810       1820       1830       1840       1850       1860
         *    *     *    *     *    *     *    *     *    *     *
ACGAGATTTC GATTCCACCG CCGCCTTCTA TGAAAGGTTG GGCTTCGGAA TCGTTTTCCG
```

FIG. 8
(CONTINUED)

```
   >NaeI
     |
  >BpmI
    | |
  >BsrFI
    | |
   NgoMI
    | |
    | 1870       1880       1890       1900       1910       1920
    * | *     *    *     *    *     *    *     *    *     *    *
GGACGCCGGC TGGATGATCC TCCAGCGCGG GGATCTCATG CTGGAGTTCT TCGCCCACCC

>BpmI                                                    >ApoI
  |                                                        |
>SV40_early_poly_A                                         |
   |                                                       |
   |    1930       1940       1950       1960       1970  |    1980
   |  *    *     *    *     *    *     *    *     *    *  |  *    *
CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA CAAATTTCAC

                       >BsmI
                         |
      1990       2000    |    2010       2020       2030       2040
    *    *     *    *    |  *    *     *    *     *    *     *    *
AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG TCCAAACTCA TCAATGTATC

                     >HincII
                        |
          >Bstl107I   >AccI
              |       | |
           >AccI    >SalI
             ||      || |
     2050    ||  2060 |     2070       2080       2090       2100
   *    *    ||*   |*  |   *    *     *    *     *    *     *    *
TTATCATGTC TGTATACCGT CGACCTCTAG CTAGAGCTTG GCGTAATCAT GGTCATAGCT
                                      _____PUC19 BACKBONE_________>

                        >BsrBI
                          |
     2110       2120      | 2130       2140       2150       2160
   *    *     *    *     *    *     *    *     *    *     *    *
GTTTCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC AACATACGAG CCGGAAGCAT
__________e__________e___PUC19 BACKBONE____e__________e__________>

                >BanI                              >AseI
                  |                                  |
     2170       2180       2190       2200       |  2210       2220
   *    *     * |  *     *    *     *    *     *   |* *    *     *    *
AAAGTGTAAA GCCTGGGGTG CCTAATGAGT GAGCTAACTC ACATTAATTG CGTTGCGCTC
__________e__________e___PUC19 BACKBONE____e__________e__________>

                                      >PvuII   >AseI     >EaeI
                                        |        |         |
     2230       2240       2250       2260    |  2270   |    2280
   *    *     *    *     *    *     *  |  *    | *    *  |  *    *
ACTGCCCGCT TTCCAGTCGG GAAACCTGTC GTGCCAGCTG CATTAATGAA TCGGCCAACG
__________e__________e___PUC19 BACKBONE____e__________e__________>
```

FIG. 8
(CONTINUED)

```
                                                  >SapI
                                                    |
                                        >HaeII    >EarI
                                          |         |
      2290        2300        2310        |  2320        2330        2340
    *    *      *    *      *    *      *  |  *    *      *    *      *    *
CGCGGGGAGA GGCGGTTTGC GTATTGGGCG CTCTTCCGCT TCCTCGCTCA CTGACTCGCT
__________e__________e___PUC19 BACKBONE____e__________e__________>

      >BsiEI                >BsrBI
        |                     |
      2350        2360        |2370        2380        2390        2400
    *   |*      *    *      *  |  *      *    *      *    *      *    *
GCGCTCGGTC GTTCGGCTGC GGCGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT
__________e__________e___PUC19 BACKBONE____e__________e__________>

                                        >AflIII
                                          |
      2410        2420        2430        |  2440        2450        2460
    *    *      *    *      *    *      *  |  *    *      *    *      *    *
ATCCACAGAA TCAGGGGATA ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC
__________e__________e___PUC19 BACKBONE____e__________e__________>

      2470        2480        2490        2500        2510        2520
    *    *      *    *      *    *      *    *      *    *      *    *
CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC CCCCTGACGA
__________e__________e___PUC19 BACKBONE____e__________e__________>

                        >DrdI
                          |
      2530        2540    |    2550        2560        2570        2580
    *    *      *    *    |  *    *      *    *      *    *      *    *
GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATA
__________e__________e___PUC19 BACKBONE____e__________e__________>

                          >BssSI                                >BsaWI
                            |                                     |
      2590        2600      |2610        2620        2630        2640
    *    *      *    *    *  |  *      *    *      *    *      *    |*
CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC
__________e__________e___PUC19 BACKBONE____e__________e__________>

                                            >HaeII              >SfcI
                                              |                   |
      2650        2660        2670        2680    |   2690      2700
    *    *      *    *      *    *      *    *    |  *    *    *  | *
CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG CTTTCTCAAT GCTCACGCTG
__________e__________e___PUC19 BACKBONE____e__________e__________>

                                                          >BsiHKAI
                                                            |
                                                >ApaLI      |
                                                  |         |
      2710        2720        2730        2740        2750  |   2760
    *    *      *    *      *    *      *    *      *  |  *  |  *    *
TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC
__________e__________e___PUC19 BACKBONE____e__________e__________>
```

FIG. 8
(CONTINUED)

```
          >BsiEI          >BsaWI
             |               |
      2770   |   2780       |2790       2800       2810       2820
    *    *   | *    *      *|   *      *    *     *    *     *    *
CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG
__________e__________e___PUC19 BACKBONE____e__________e__________>

                                >AlwNI
                                  |
      2830       2840       2850       2860       2870       2880
    *    *     *    *     *    |*     *    *     *    *     *    *
ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT
__________e__________e___PUC19 BACKBONE____e__________e__________>

      >SfcI
        |
      2890       2900       2910       2920       2930       2940
    *   |*     *    *     *    *     *    *     *    *     *    *
AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT
__________e__________e___PUC19 BACKBONE____e__________e__________>

                                          >Eco57I
                                             |
      2950       2960       2970       2980  |     2990       3000
    *    *     *    *     *    *     *    *  |  *    *     *    *
ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG
__________e__________e___PUC19 BACKBONE____e__________e__________>

      3010       3020       3030       3040       3050       3060
    *    *     *    *     *    *     *    *     *    *     *    *
ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC
__________e__________e___PUC19 BACKBONE____e__________e__________>

      3070       3080       3090       3100       3110       3120
    *    *     *    *     *    *     *    *     *    *     *    *
GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA
__________e__________e___PUC19 BACKBONE____e__________e__________>

                                      >BspHI
                                        |
      3130       3140       3150       |   3160       3170       3180
    *    *     *    *     *    *       | *    *     *    *     *    *
GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA TTATCAAAAA GGATCTTCAC
__________e__________e___PUC19 BACKBONE____e__________e__________>

          >DraI                   >DraI
            |                       |
      3190  |     3200       3210  |     3220       3230       3240
    *    *  | *    *     *    *    |  *    *     *    *     *    *
CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC
__________e__________e___PUC19 BACKBONE____e__________e__________>

                                      >BanI
                                        |
      3250       3260       3270       |  3280       3290       3300
    *    *     *    *     *    *       |*    *     *    *     *    *
TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT
                ________a__________a__AMP-ORF_a__________a__________>
__________e__________e___PUC19 BACKBONE____e__________e__________>
```

FIG. 8
(CONTINUED)

```
                                >AhdI
                                  |
      3310        3320          |3330        3340        3350        3360
    *    *      *    *      *    |   *     *    *     *    *     *    *
TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT
__________a__________a_____AMP-ORF_________a__________a__________>
__________e__________e___PUC19 BACKBONE____e__________e__________>

                               >BsaI
                                 |
                               >BsrDI     >BpmI       >BsrFI
                                 |          |           |
      3370        3380          3390       |3400       |3410        3420
    *    *      *    *      *    |   *     *|   *     *|   *     *    *
ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT
__________a__________a_____AMP-ORF_________a__________a__________>
__________e__________e___PUC19 BACKBONE____e__________e__________>

                               >BglI
                                 |
      3430        3440          |3450        3460        3470        3480
    *    *      *    *      *    |   *     *    *     *    *     *    *
ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC
__________a__________a_____AMP-ORF_________a__________a__________>
__________e__________e___PUC19 BACKBONE____e__________e__________>

                    >AseI
                      |
      3490        3500          3510        3520        3530        3540
    *    *      *   | *      *    *     *    *     *    *     *    *
CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA
__________a__________a_____AMP-ORF_________a__________a__________>
__________e__________e___PUC19 BACKBONE____e__________e__________>

          >Psp1406I
             |
       >FspI |      >BsrDI   >SfcI       >MslI
         |   |        |        |          |
        3550 |     3560 |     3570       3580        3590        3600
      *  | *  |  *    *  |  *  |  *     *  | *     *    *     *    *
TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG
__________a__________a_____AMP-ORF_________a__________a__________>
__________e__________e___PUC19 BACKBONE____e__________e__________>

                    >BsaWI
                      |
      3610        3620          3630        3640        3650        3660
    *    *      *   | *      *    *     *    *     *    *     *    *
TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT
__________a__________a_____AMP-ORF_________a__________a__________>
__________e__________e___PUC19 BACKBONE____e__________e__________>

                                    >BsiEI
                                      |
                                    >PvuI                  >EaeI
                                      |                      |
      3670        3680          3690        |3700       3710     |  3720
    *    *      *    *      *    *     *     |   *     *    *     |*    *
GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC
__________a__________a_____AMP-ORF_________a__________a__________>
__________e__________e___PUC19 BACKBONE____e__________e__________>
```

FIG. 8
(CONTINUED)

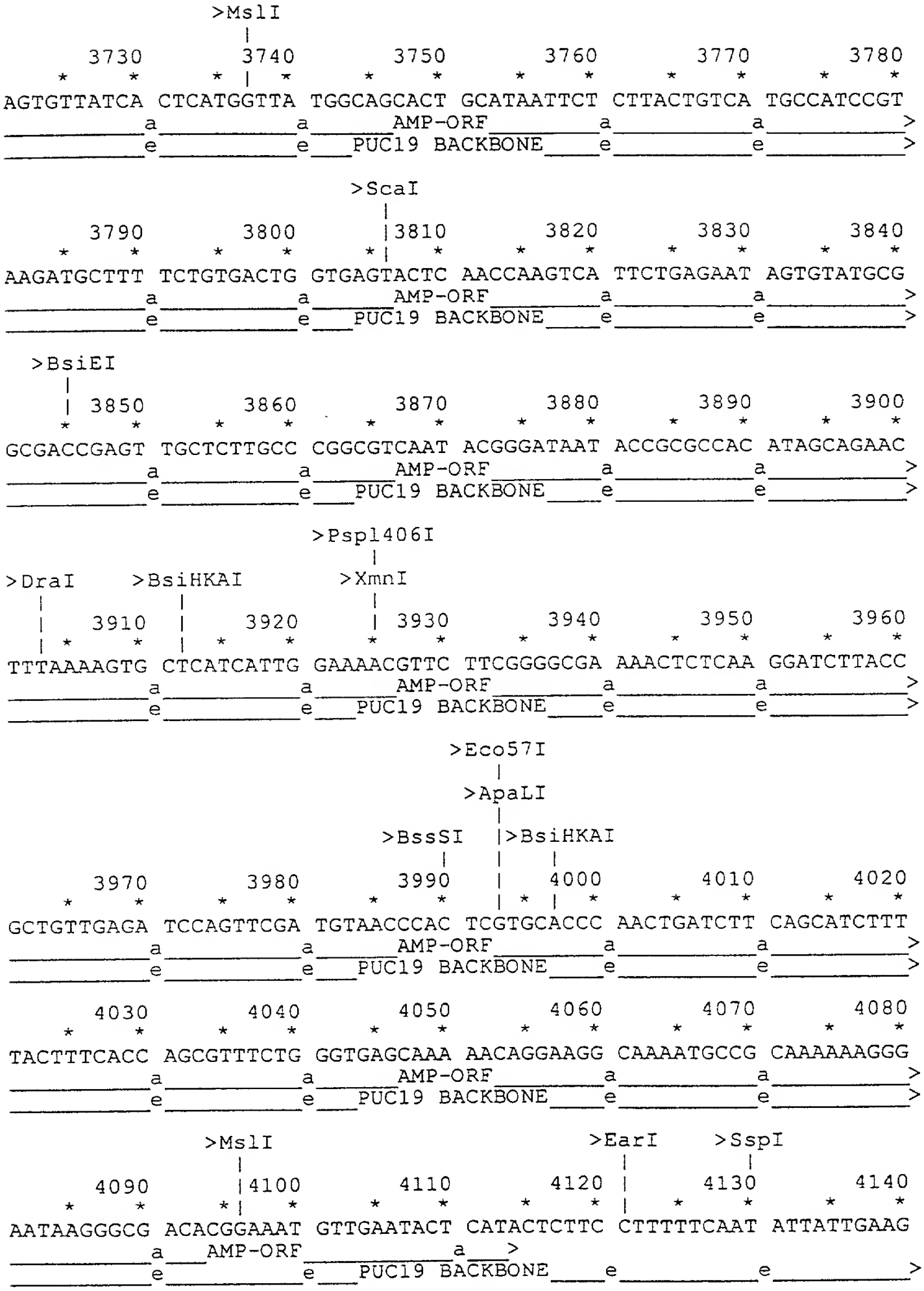


FIG. 8
(CONTINUED)

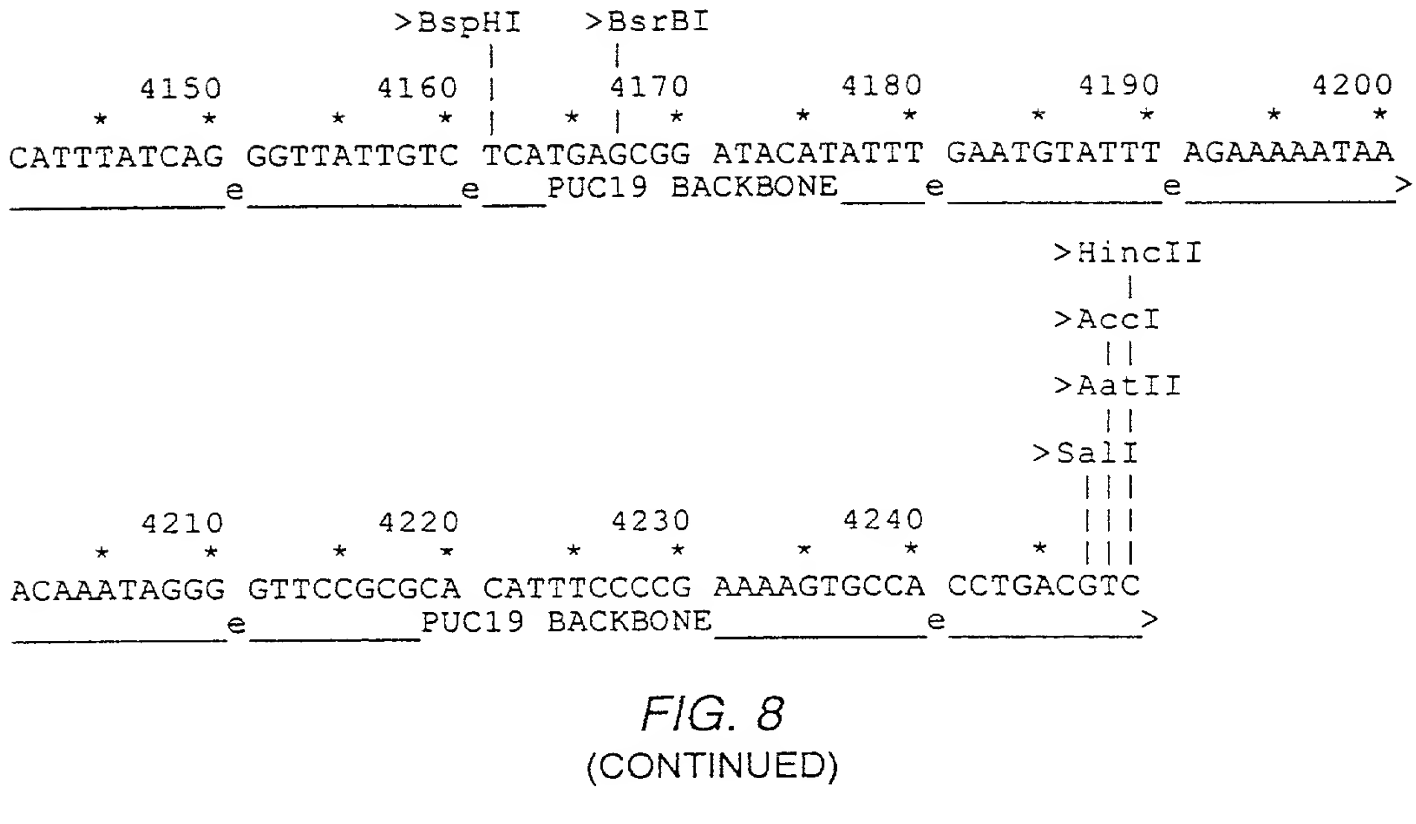


FIG. 8
(CONTINUED)

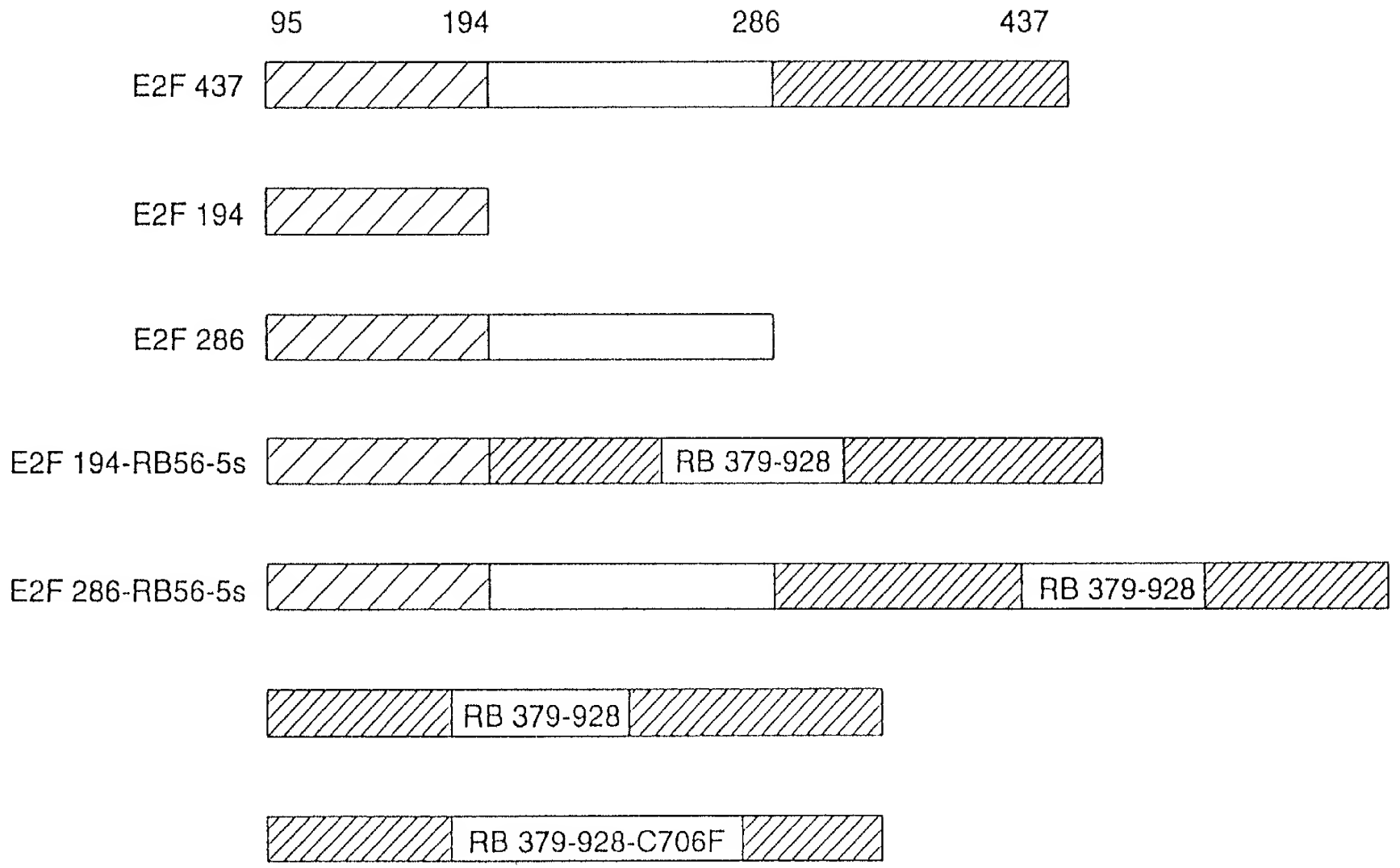

95
194
286
437
E2F 437
E2F 194
E2F 286
E2F 194-RB56-5s
RB 379-928
E2F 286-RB56-5s
RB 379-928
RB 379-928
RB 379-928-C706F

FIG. 9

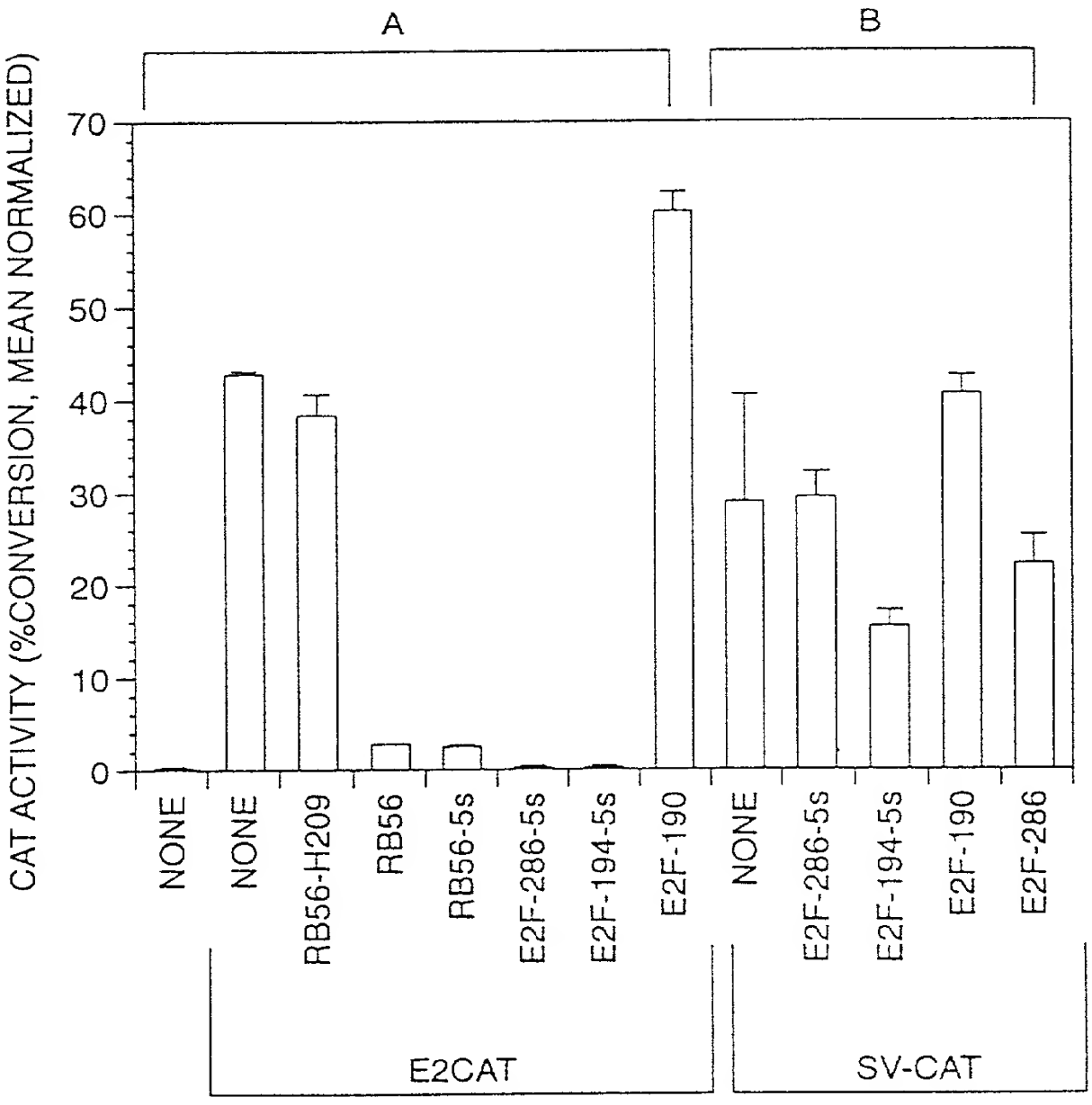

A
B
CAT ACTIVITY (%CONVERSION, MEAN NORMALIZED)
70
60
50
40
30
20
10
0
NONE
NONE
RB56-H209
RB56
RB56-5s
E2F-286-5s
E2F-194-5s
E2F-190
NONE
E2F-286-5s
E2F-194-5s
E2F-190
E2F-286
E2CAT
SV-CAT

FIG. 10

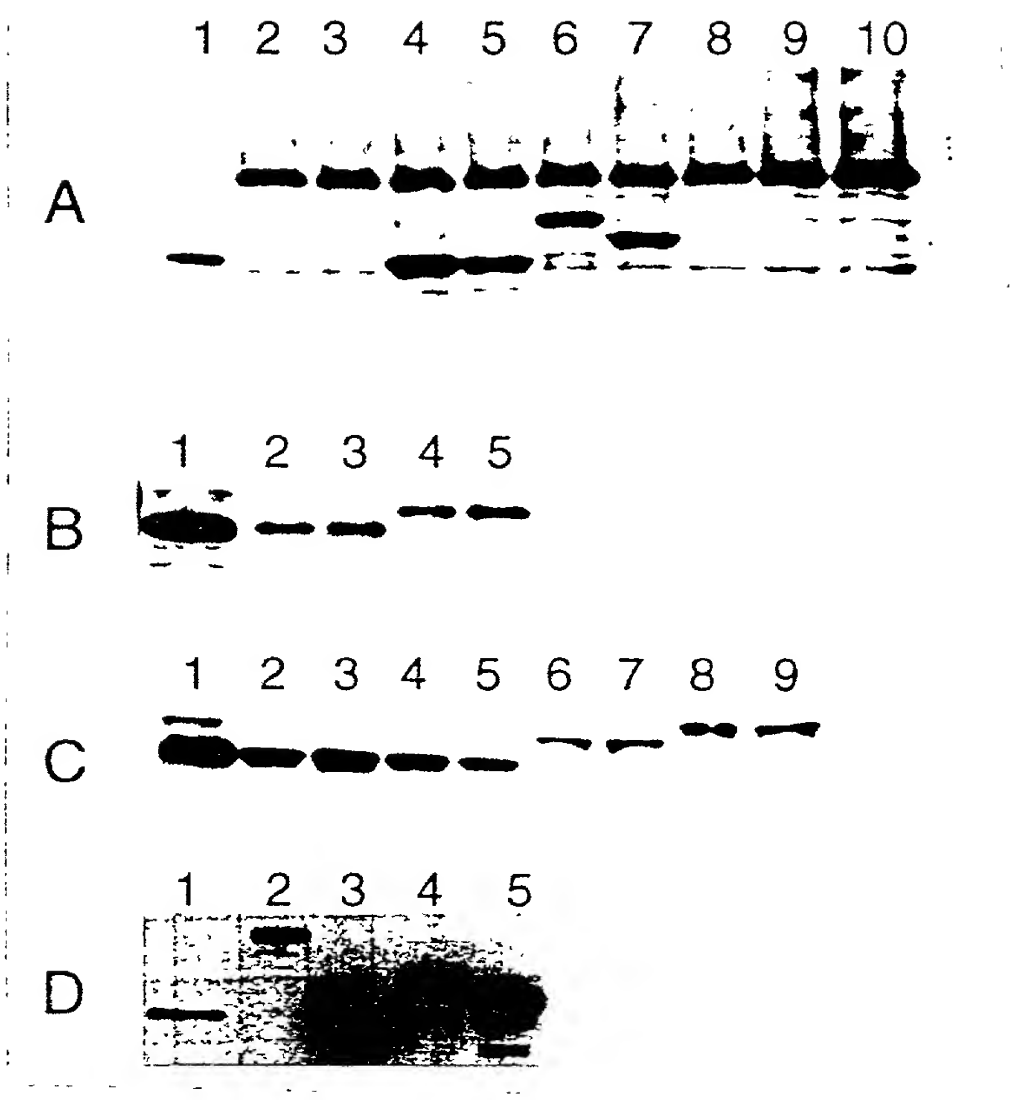


FIG. 11

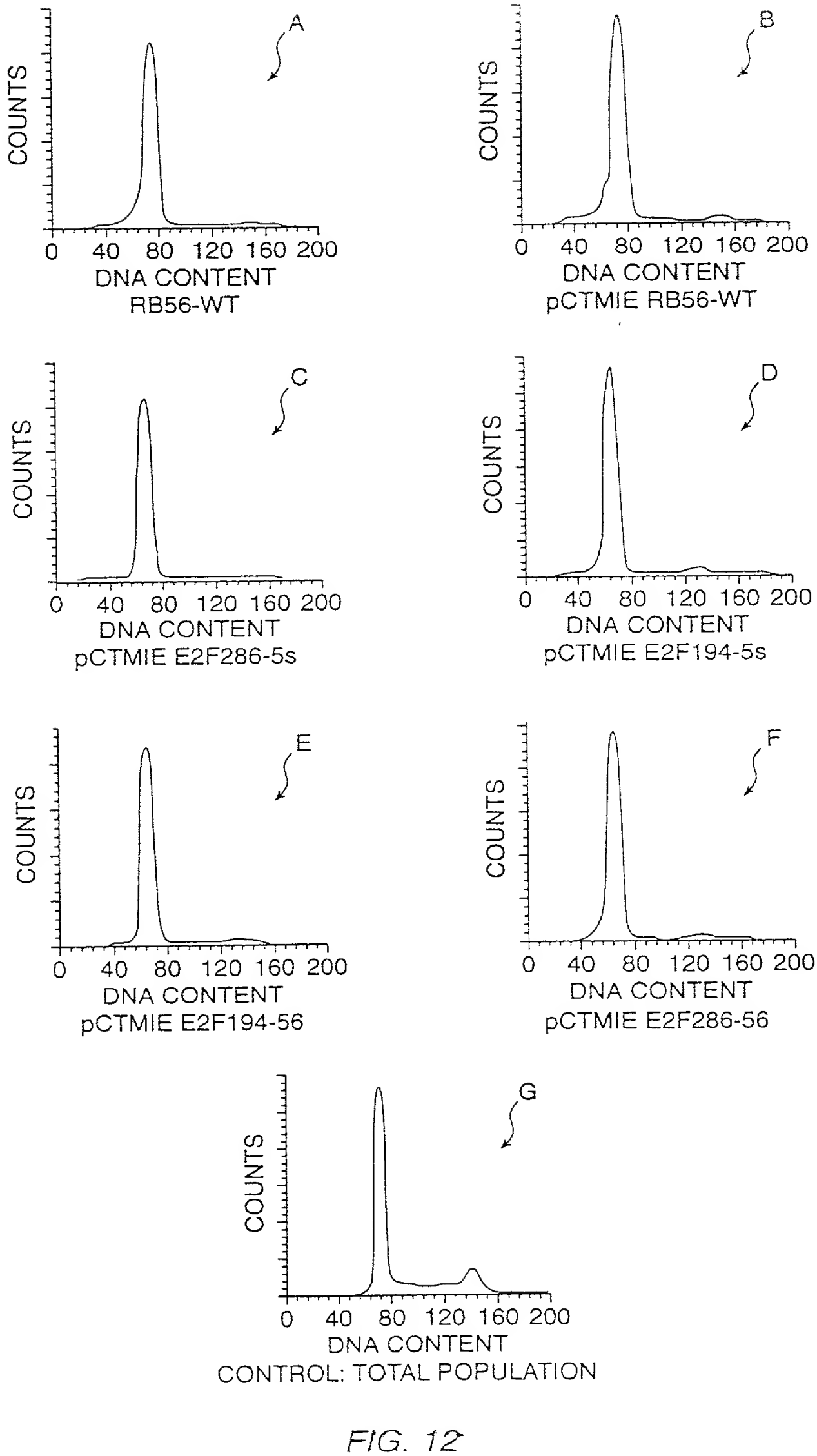

A
COUNTS
0 40 80 120 160 200
DNA CONTENT
RB56-WT
B
COUNTS
0 40 80 120 160 200
DNA CONTENT
pCTMIE RB56-WT
C
COUNTS
0 40 80 120 160 200
DNA CONTENT
pCTMIE E2F286-5s
D
COUNTS
0 40 80 120 160 200
DNA CONTENT
pCTMIE E2F194-5s
E
COUNTS
0 40 80 120 160 200
DNA CONTENT
pCTMIE E2F194-56
F
COUNTS
0 40 80 120 160 200
DNA CONTENT
pCTMIE E2F286-56
G
COUNTS
0 40 80 120 160 200
DNA CONTENT
CONTROL: TOTAL POPULATION

FIG. 12

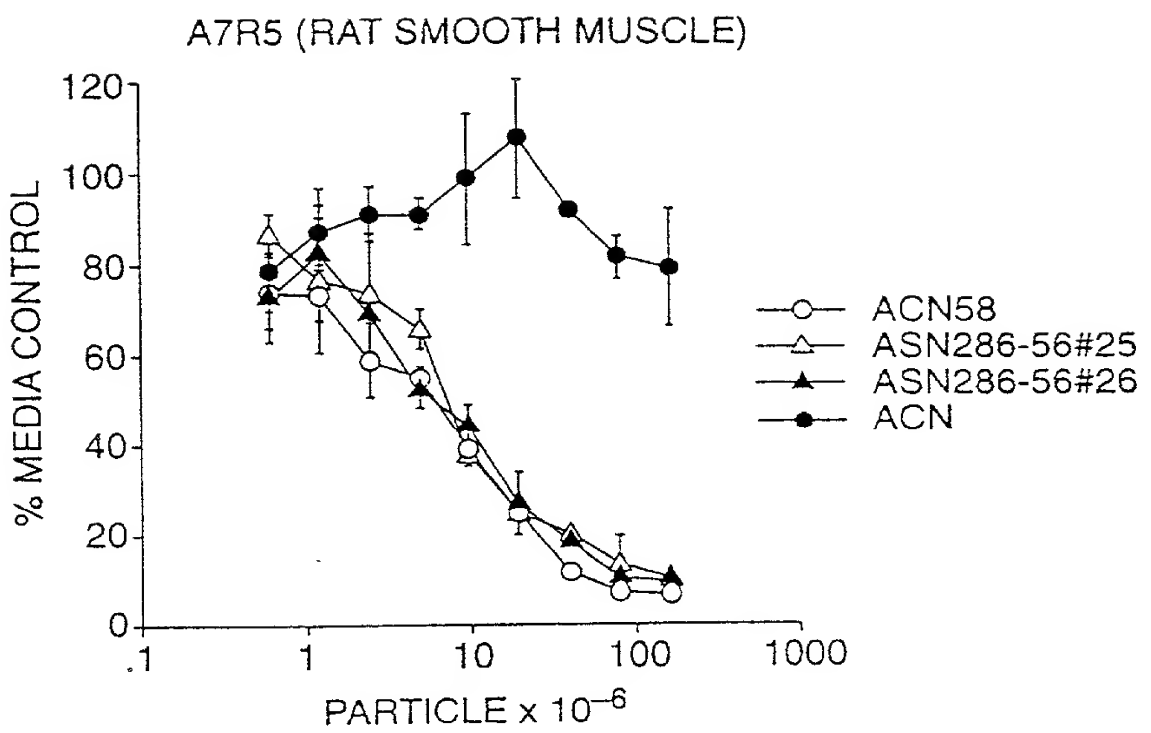


FIG. 13A

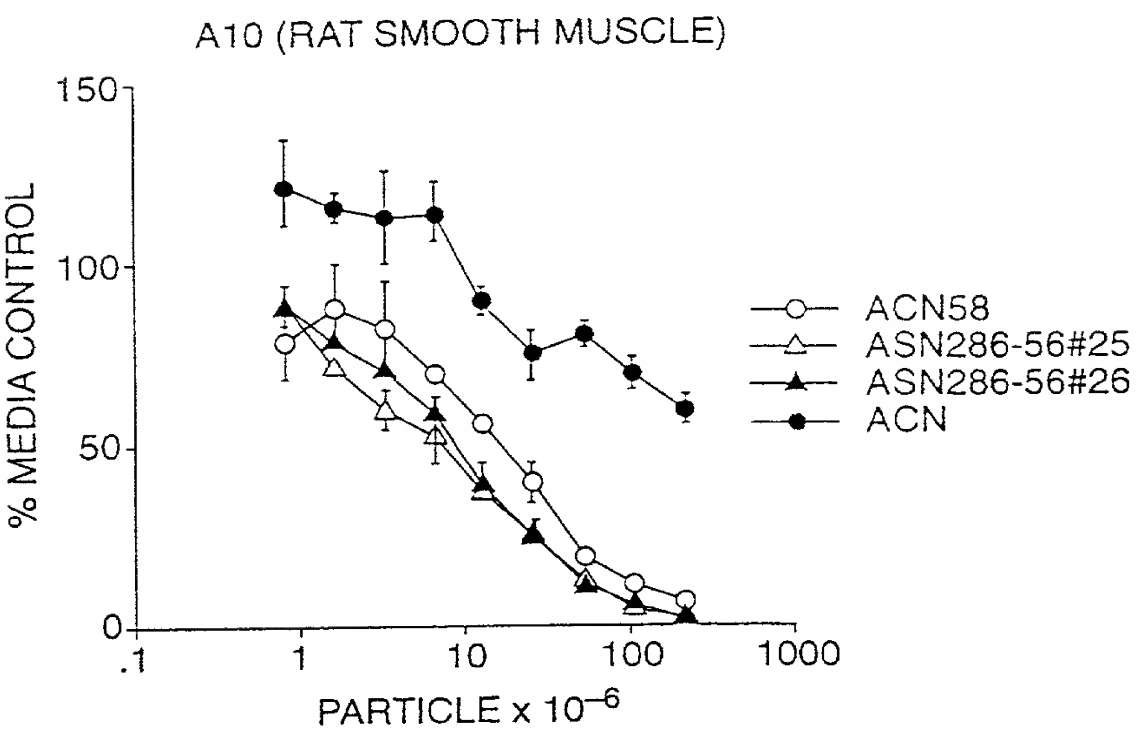


FIG. 13B

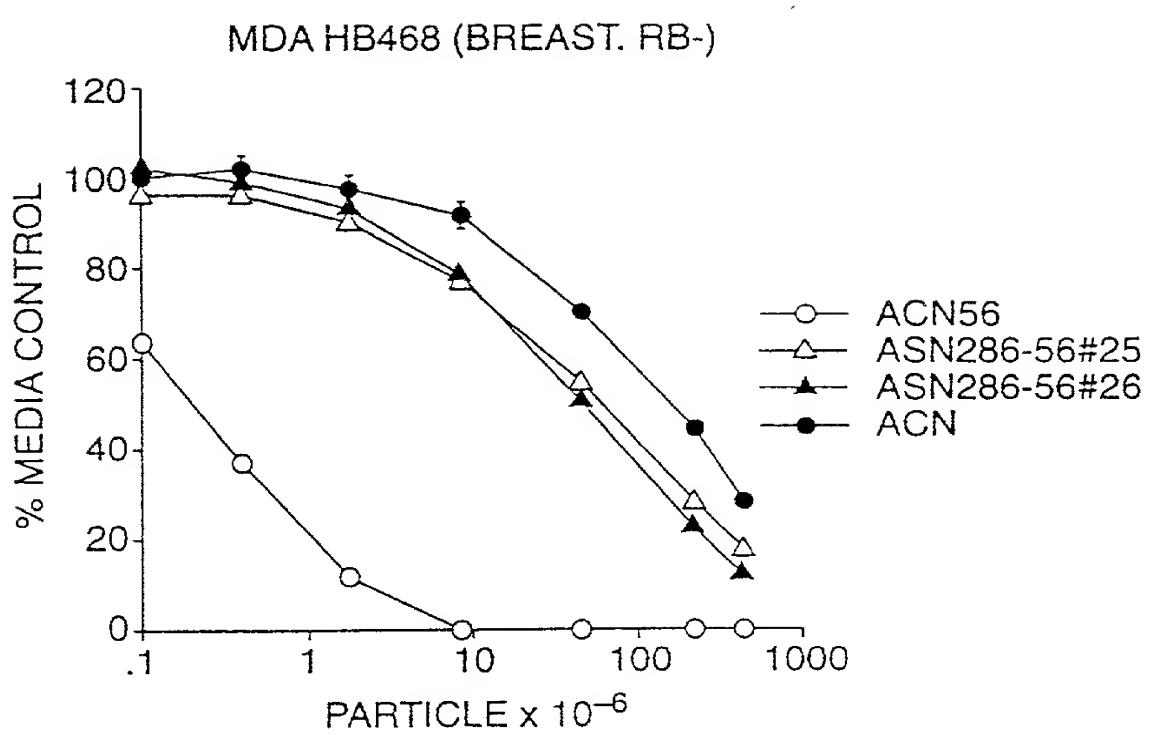


FIG. 14A

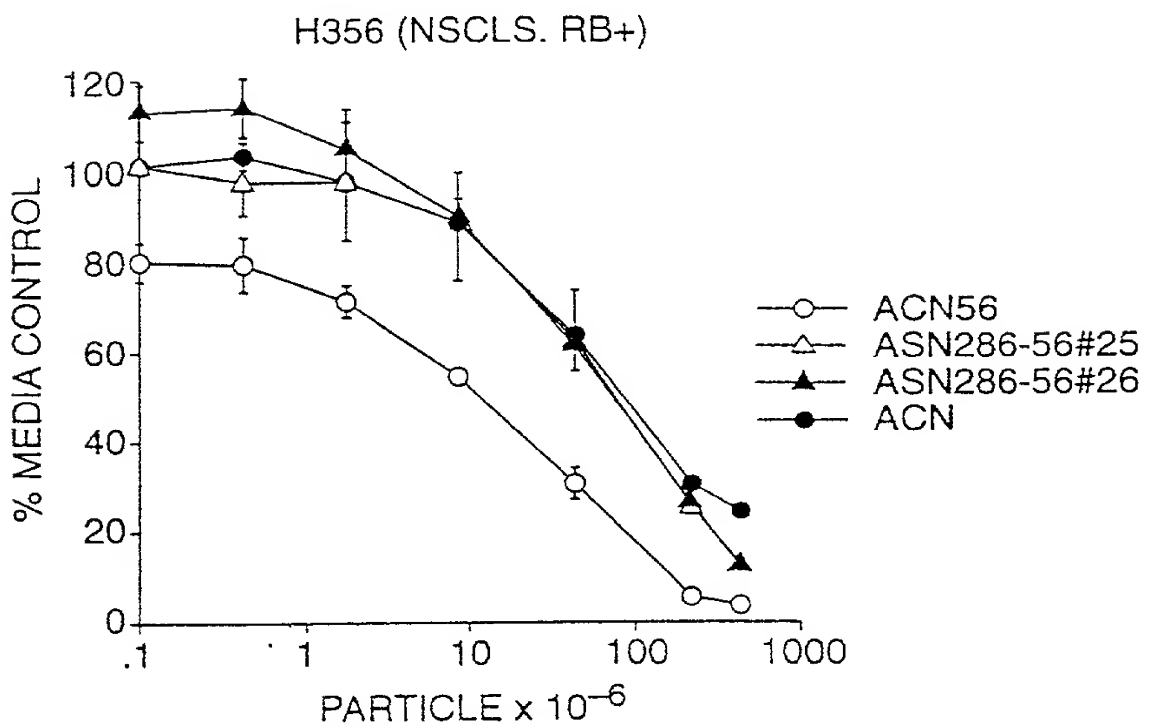


FIG. 14B

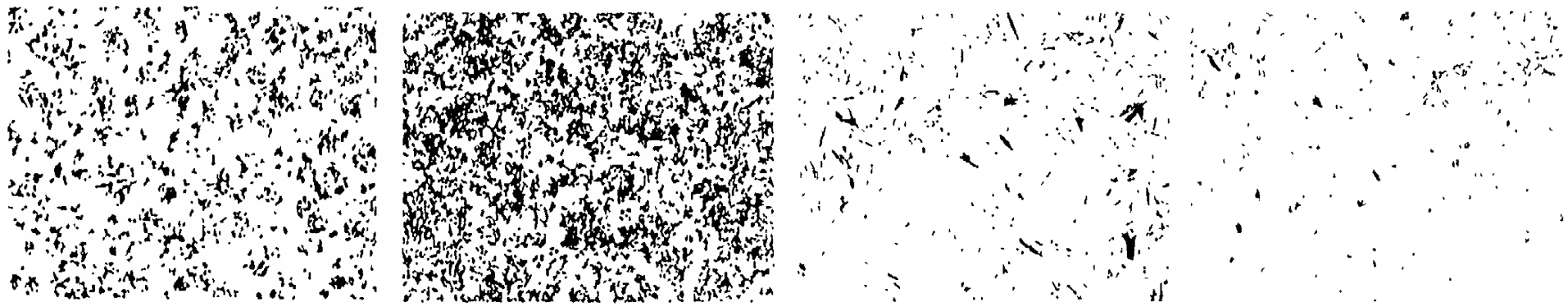

H358
MB468
A7R5
A10
Ad-BGL MOI=5
H358 MB468 --A7R5-- --A10--
ACN56
MOI: 50 50 50 250 100 500

FIG. 15

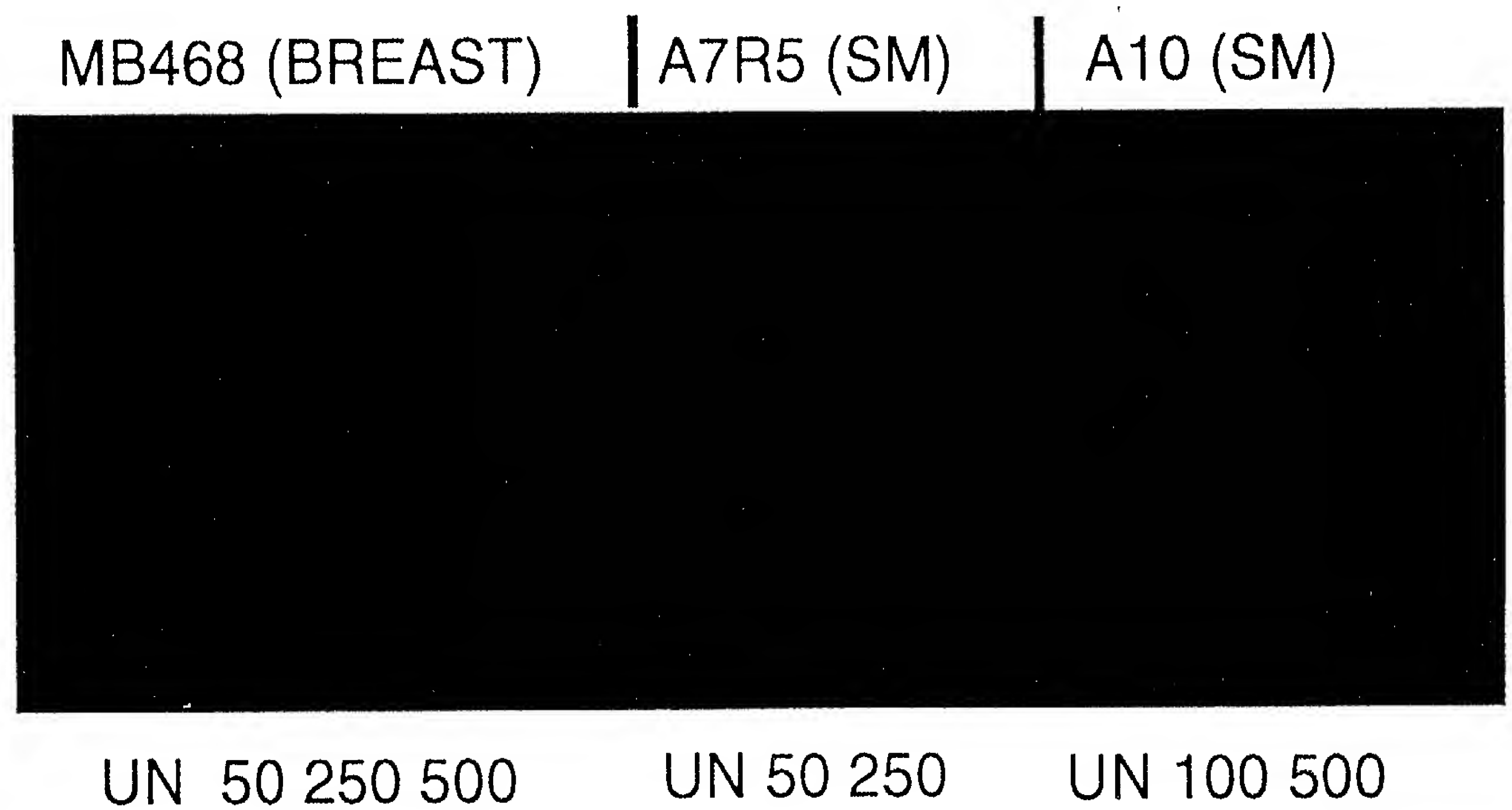


FIG. 16

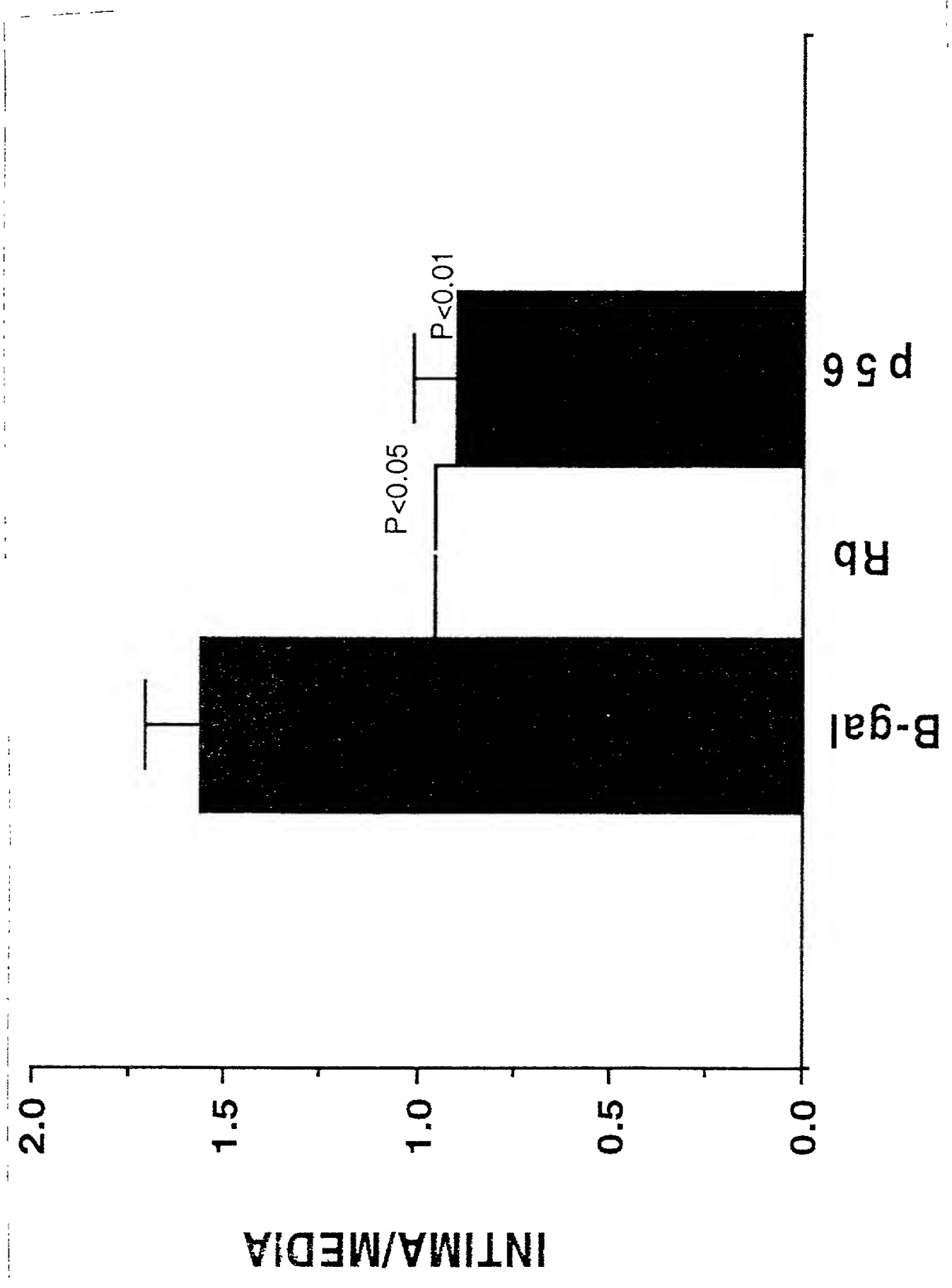


FIG. 17

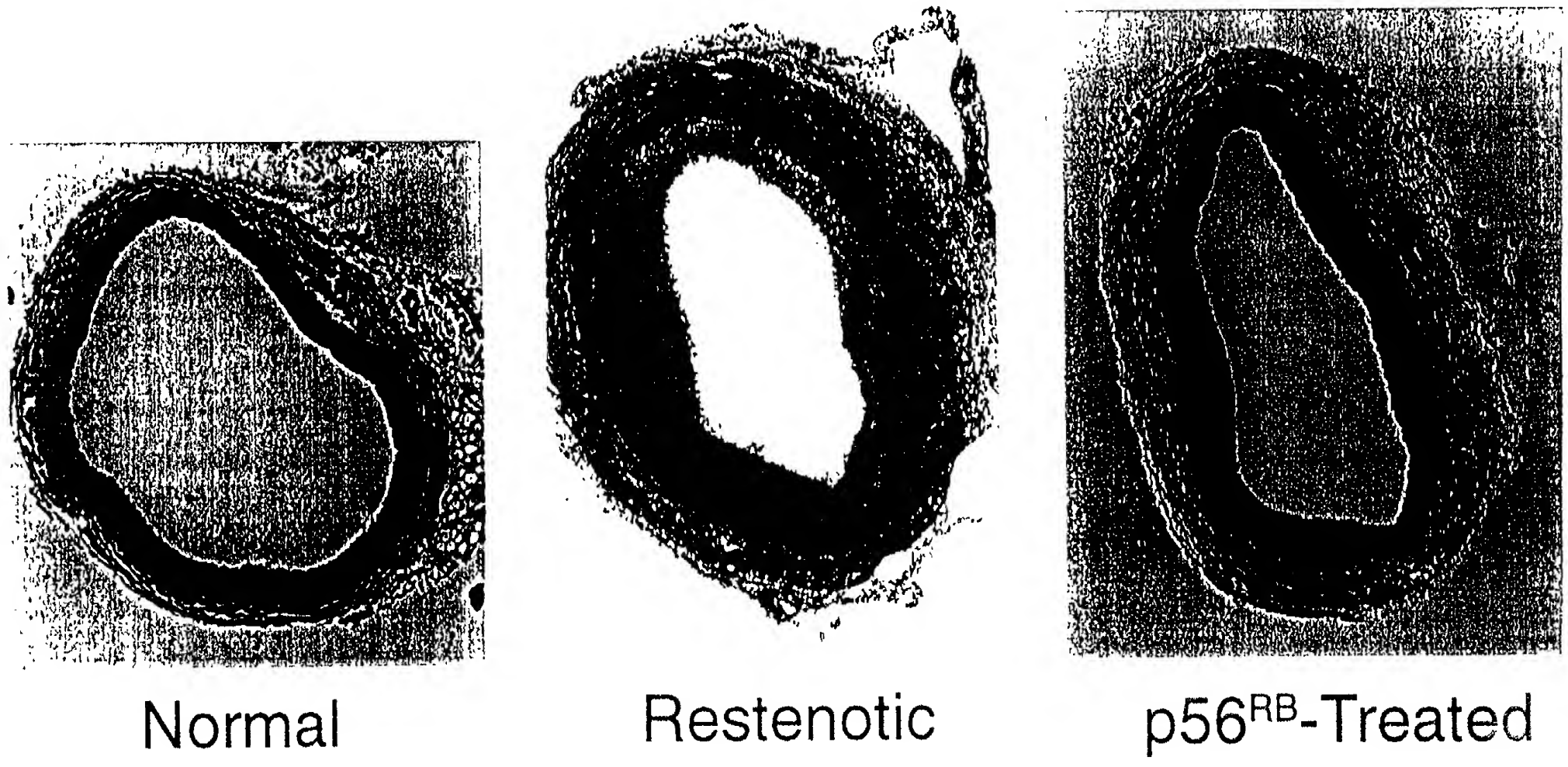


FIG. 18

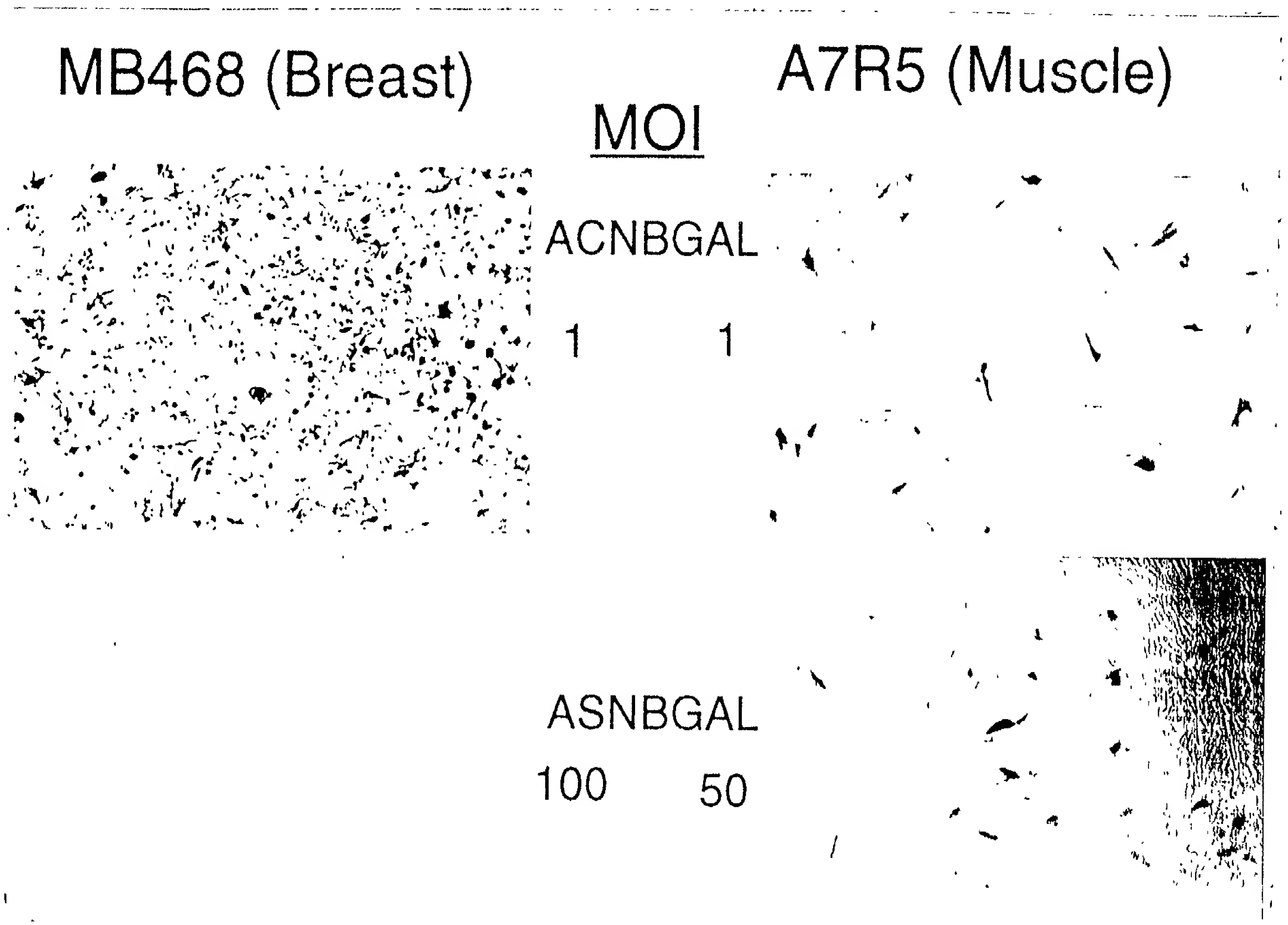


FIG. 19

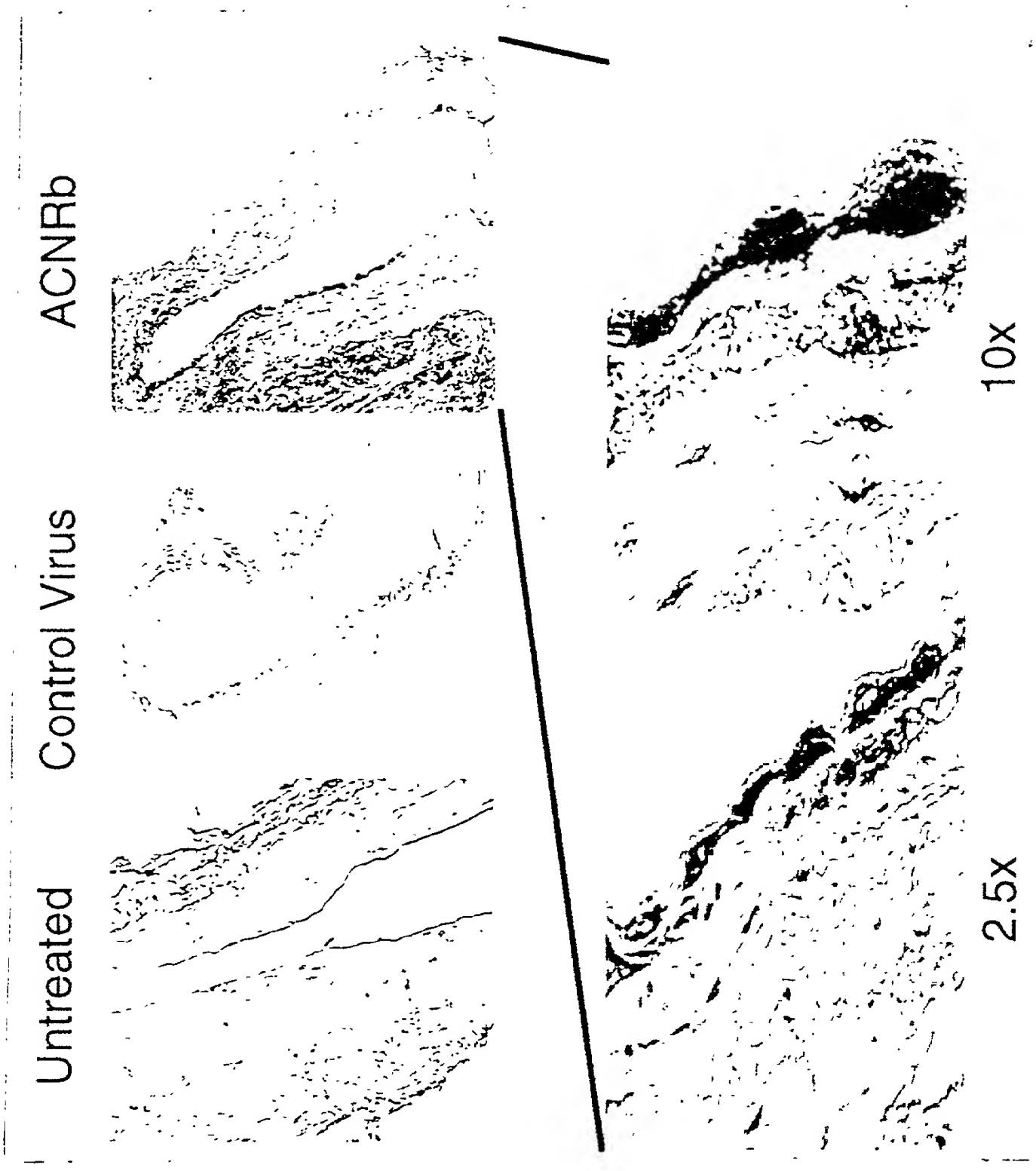


FIG. 20

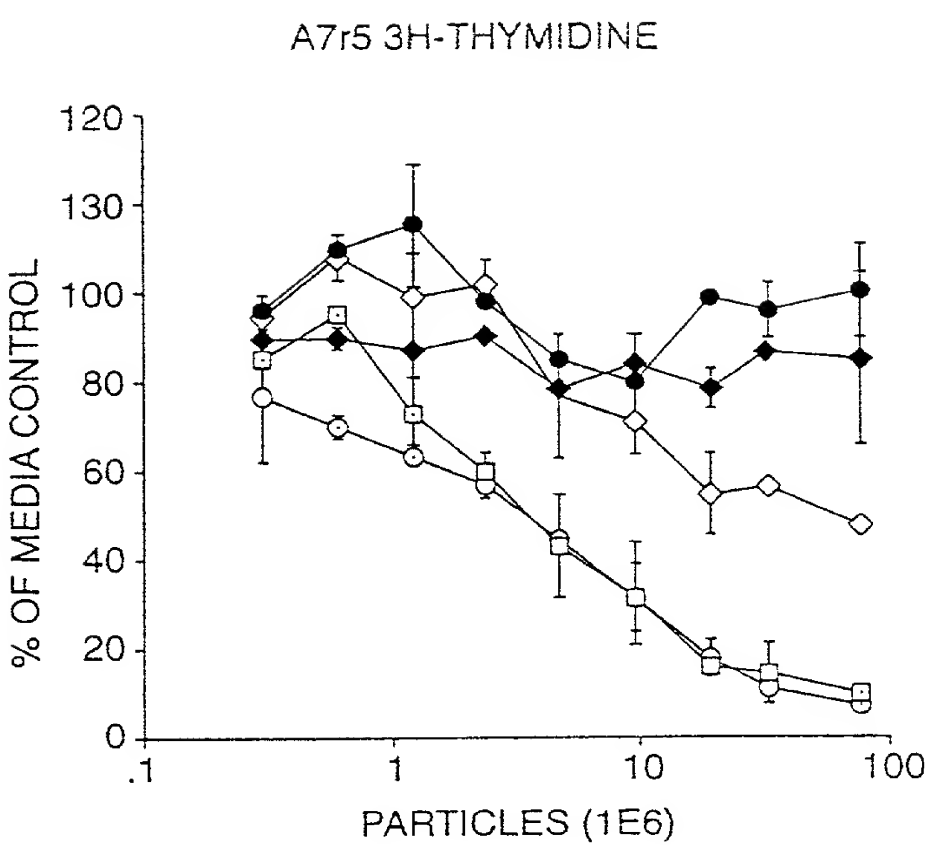


FIG. 21A

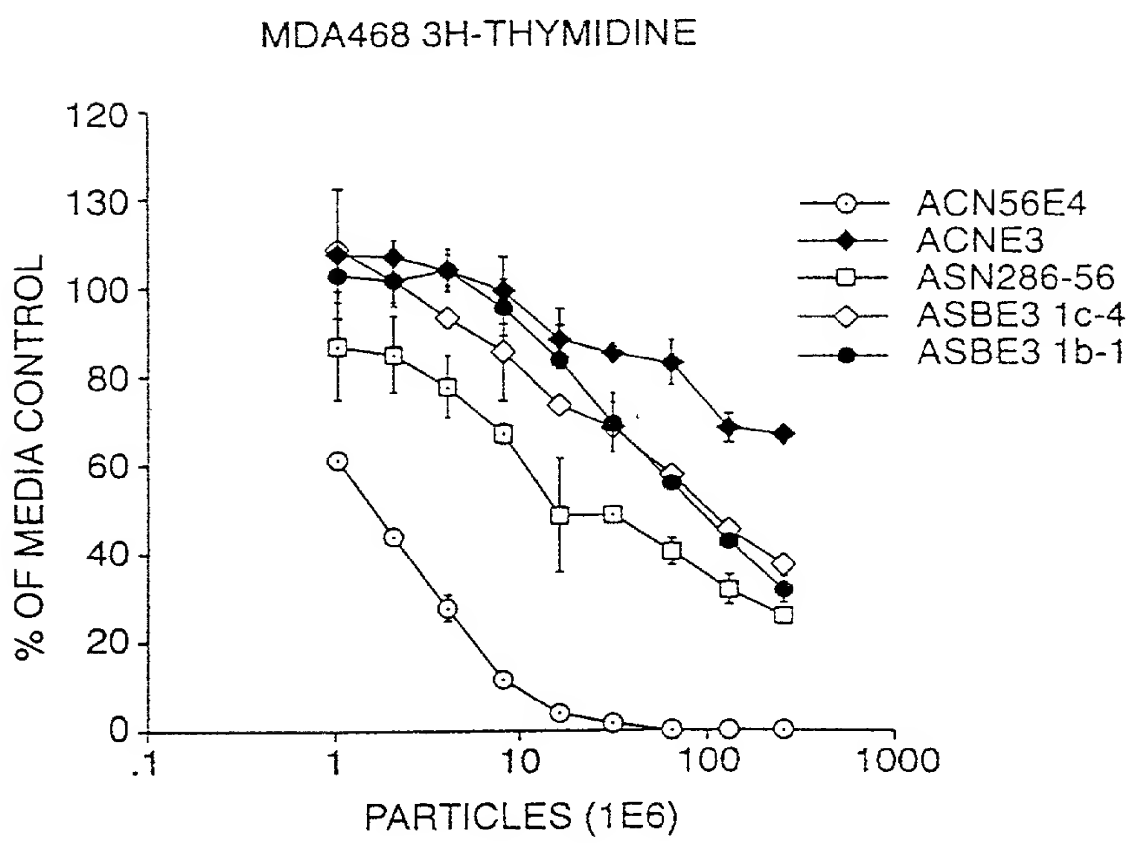


FIG. 21B

Inventor: Douglas Antelman *et al.*
Assignee: CANJI, INC.
Title: METHODS OF TREATING HYPERPROLIFERATIVE DISORDERS USING RETINOBLASTOMA FUSION PROTEINS
Ser.No.:
Filed: Herewith
Our File: 16930-0010-22
Townsend and Townsend and Crew LLP
San Francisco, CA 94111
(415) 576-0200

Attorney Docket No. 16930-001020

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: **TISSUE SPECIFIC EXPRESSION OF RETINOBLASTOMA PROTEIN** the specification of which ___ is attached hereto or _X_ was filed on February 14, 1997 as Application No. 08/801,092 and was amended on ___ (if applicable).

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56. I claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign applications(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Country	Application No.	Date of Filing	Priority Claimed Under 35 USC 119
			Yes _ No _
			Yes _ No _

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date

I claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Date of Filing	Status
08/751,517	11/15/96	_ Patented _X_ Pending _ Abandoned
		_ Patented _ Pending _ Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

William M. Smith, Reg. No. 30,223
James M. Heslin, Reg. No. 29,541
Renee A. Fitts, Reg. No. 35,136
Randolph T. Apple, Reg. No. 36,429
Joe Liebeschuetz, Reg. No. 37,505
Karen B. Dow, Reg. No. 29,684

Send Correspondence to:	Direct Telephone Calls to: (Name, Reg. No., Telephone No.)
William M. Smith **TOWNSEND and TOWNSEND and CREW LLP** **Two Embarcadero Center, 8th Floor** **San Francisco, CA 94111-3834**	**Name: William M. Smith** **Reg. No.: 30,223** **Telephone: (415) 326-2400**

Full Name of Inventor 1	Last Name **ANTELMAN**	First Name **DOUGLAS**	Middle Name or Initial	
Residence & Citizenship	City **ENCINITAS**	State/Foreign Country **CALIFORNIA**	Country of Citizenship **U.S.A.**	
Post Office Address	Post Office Address **1716 SWALLOWTAIL ROAD**	City **ENCINITAS**	State/Country **CA**	Zip Code **92024**
Full Name of Inventor 2	Last Name **GREGORY**	First Name **RICHARD**	Middle Name or Initial **J.**	
Residence & Citizenship	City **WESTFORD**	State/Foreign Country **MASSACHUSETTS**	Country of Citizenship **U.S.A.**	
Post Office Address	Post Office Address **2 WINTERGREEN LANE**	City **WESTFORD**	State/Country **MA**	Zip Code **01886**
Full Name of Inventor 3	Last Name **WILLS**	First Name **KENNETH**	Middle Name or Initial **N.**	
Residence & Citizenship	City **ENCINITAS**	State/Foreign Country **CALIFORNIA**	Country of Citizenship **U.S.A.**	
Post Office Address	Post Office Address **821 BLUFFCREST LANE**	City **ENCINITAS**	State/Country **CA**	Zip Code **92024**

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1	Signature of Inventor 2	Signature of Inventor 3
D. Antelman DOUGLAS ANTELMAN	RICHARD J. GREGORY	Kenneth Wills KENNETH N. WILLS
Date April 25 1997	Date	Date April 25, 1997

DP.MRG 8/96
H:\HOME\RAF\WORK\16930\1020\1020.DEC

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 1	Signature of Inventor 2	Signature of Inventor 3
DOUGLAS ANTELMAN	Richard J Gregory **RICHARD J. GREGORY**	**KENNETH N. WILLS**
Date	Date 4/23/97	Date

DP.MRG 8/96
H:\HOME\RAF\WORK\16930\1020\1020.DEC

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Antelman, Douglas
Gregory, Richard J.
Wils, Kenneth N.

(ii) TITLE OF INVENTION: Tissue Specific Expression of Retinoblastoma Protein

(iii) NUMBER OF SEQUENCES: 46

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: TOWNSEND and TOWNSEND and CREW LLP
(B) STREET: Two Embarcadero Center, 8th Floor
(C) CITY: San Francisco
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94111

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/801,092
(B) FILING DATE: 14-FEB-1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/751,517
(B) FILING DATE: 15-NOV-1996
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Fitts, Renee A.
(B) REGISTRATION NUMBER: 35,136
(C) REFERENCE/DOCKET NUMBER: 016930-001020

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415-576-0200
(B) TELEFAX: 703-576-0300

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 437 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Ala Leu Ala Gly Ala Pro Ala Gly Gly Pro Cys Ala Pro Ala Leu
1               5                  10                  15

Glu Ala Leu Leu Gly Ala Gly Ala Leu Arg Leu Leu Asp Ser Ser Gln
            20                  25                  30

Ile Val Ile Ile Ser Ala Ala Gln Asp Ala Ser Ala Pro Pro Ala Pro
        35                  40                  45

Thr Gly Pro Ala Ala Pro Ala Ala Gly Pro Cys Asp Pro Asp Leu Leu
    50                  55                  60

Leu Phe Ala Thr Pro Gln Ala Pro Arg Pro Thr Pro Ser Ala Pro Arg
65                  70                  75                  80

Pro Ala Leu Gly Arg Pro Pro Val Lys Arg Arg Leu Asp Leu Glu Thr
                85                  90                  95

Asp His Gln Tyr Leu Ala Glu Ser Ser Gly Pro Ala Arg Gly Arg Gly
            100                 105                 110

Arg His Pro Gly Lys Gly Val Lys Ser Pro Gly Glu Lys Ser Arg Tyr
        115                 120                 125

Glu Thr Ser Leu Asn Leu Thr Thr Lys Arg Phe Leu Glu Leu Leu Ser
    130                 135                 140

His Ser Ala Asp Gly Val Val Asp Leu Asn Trp Ala Ala Glu Val Leu
145                 150                 155                 160

Lys Val Gln Lys Arg Arg Ile Tyr Asp Ile Thr Asn Val Leu Glu Gly
                165                 170                 175

Ile Gln Leu Ile Ala Lys Lys Ser Lys Asn His Ile Gln Trp Leu Gly
            180                 185                 190

Ser His Thr Thr Val Gly Val Gly Gly Arg Leu Glu Gly Leu Thr Gln
        195                 200                 205

Asp Leu Arg Gln Leu Gln Glu Ser Glu Gln Gln Leu Asp His Leu Met
    210                 215                 220

Asn Ile Cys Thr Thr Gln Leu Arg Leu Leu Ser Glu Asp Thr Asp Ser
225                 230                 235                 240

Gln Arg Leu Ala Tyr Val Thr Cys Gln Asp Leu Arg Ser Ile Ala Asp
                245                 250                 255

Pro Ala Glu Gln Met Val Met Val Ile Lys Ala Pro Pro Glu Thr Gln
            260                 265                 270

Leu Gln Ala Val Asp Ser Ser Glu Asn Phe Gln Ile Ser Leu Lys Ser
        275                 280                 285

Lys Gln Gly Pro Ile Asp Val Phe Leu Cys Pro Glu Glu Thr Val Gly
    290                 295                 300

Gly Ile Ser Pro Gly Lys Thr Pro Ser Gln Glu Val Thr Ser Glu Glu
305                 310                 315                 320

Glu Asn Arg Ala Thr Asp Ser Ala Thr Ile Val Ser Pro Pro Pro Ser
                325                 330                 335
```

Ser Pro Pro Ser Ser Leu Thr Thr Asp Pro Ser Gln Ser Leu Leu Ser
340 345 350

Leu Glu Gln Glu Pro Leu Leu Ser Arg Met Gly Ser Leu Arg Ala Pro
355 360 365

Val Asp Glu Asp Arg Leu Ser Pro Leu Val Ala Ala Asp Ser Leu Leu
370 375 380

Glu His Val Arg Glu Asp Phe Ser Gly Leu Leu Pro Glu Glu Phe Ile
385 390 395 400

Ser Leu Ser Pro Pro His Glu Ala Leu Asp Tyr His Phe Gly Leu Glu
405 410 415

Glu Gly Glu Gly Ile Arg Asp Leu Phe Asp Cys Asp Phe Gly Asp Leu
420 425 430

Thr Pro Leu Asp Phe
435

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2517 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGAATTCCGT GGCCGGGACT TTGCAGGCAG CGGCGGCCGG GGGCGGAGCG GGATCGAGCC 60

CTCGCCGAGG CCTGCCGCCA TGGGCCCGCG CCGCCGCCGC CGCCTGTCAC CCGGGCCGCG 120

CGGGCCGTGA GCGTCATGGC CTTGGCCGGG GCCCCTGCGG GCGGCCCATG CGCGCCGGCG 180

CTGGAGGCCC TGCTCGGGGC CGGCGCGCTG CGGCTGCTCG ACTCCTCGCA GATCGTCATC 240

ATCTCCGCCG CGCAGGACGC CAGCGCCCCG CCGGCTCCCA CCGGCCCCGC GGCGCCCGCC 300

GCCGGCCCCT GCGACCCTGA CCTGCTGCTC TTCGCCACAC CGCAGGCGCC CCGGCCCACA 360

CCCAGTGCGC CGCGGCCCGC GCTCGGCCGC CCGCCGGTGA AGCGGAGGCT GGACCTGGAA 420

ACTGACCATC AGTACCTGGC CGAGAGCAGT GGGCCAGCTC GGGGCAGAGG CCGCCATCCA 480

GGAAAAGGTG TGAAATCCCC GGGGGAGAAG TCACGCTATG AGACCTCACT GAATCTGACC 540

ACCAAGCGCT TCCTGGAGCT GCTGAGCCAC TCGGCTGACG GTGTCGTCGA CCTGAACTGG 600

GCTGCCGAGG TGCTGAAGGT GCAGAAGCGG CGCATCTATG ACATCACCAA CGTCCTTGAG 660

GGCATCCAGC TCATTGCCAA GAAGTCCAAG AACCACATCC AGTGGCTGGG CAGCCACACC 720

ACAGTGGGCG TCGGCGGACG GCTTGAGGGG TTGACCCAGG ACCTCCGACA GCTGCAGGAG 780

AGCGAGCAGC AGCTGGACCA CCTGATGAAT ATCTGTACTA CGCAGCTGCG CCTGCTCTCC 840

GAGGACACTG ACAGCCAGCG CCTGGCCTAC GTGACGTGTC AGGACCTTCG TAGCATTGCA 900

GACCCTGCAG AGCAGATGGT TATGGTGATC AAAGCCCCTC CTGAGACCCA GCTCCAAGCC 960

GTGGACTCTT CGGAGAACTT TCAGATCTCC CTTAAGAGCA AACAAGGCCC GATCGATGTT 1020

TTCCTGTGCC CTGAGGAGAC CGTAGGTGGG ATCAGCCCTG GGAAGACCCC ATCCCAGGAG 1080

GTCACTTCTG AGGAGGAGAA CAGGGCCACT GACTCTGCCA CCATAGTGTC ACCACCACCA 1140

TCATCTCCCC CCTCATCCCT CACCACAGAT CCCAGCCAGT CTCTACTCAG CCTGGAGCAA 1200

GAACCGCTGT TGTCCCGGAT GGGCAGCCTG CGGGCTCCCG TGGACGAGGA CCGCCTGTCC 1260

CCGCTGGTGG CGGCCGACTC GCTCCTGGAG CATGTGCGGG AGGACTTCTC CGGCCTCCTC 1320

CCTGAGGAGT TCATCAGCCT TTCCCCACCC CACGAGGCCC TCGACTACCA CTTCGGCCTC 1380

GAGGAGGGCG AGGGCATCAG AGACCTCTTC GACTGTGACT TTGGGGACCT CACCCCCCTG 1440

GATTTCTGAC AGGGCTTGGA GGGACCAGGG TTTCCAGAGT AGCTCACCTT GTCTCTGCAG 1500

CCCTGGAGCC CCCTGTCCCT GGCCGTCCTC CCAGCCTGTT TGGAAACATT TAATTTATAC 1560

CCCTCTCCTC TGTCTCCAGA AGCTTCTAGC TCTGGGGTCT GGCTACCGCT AGGAGGCTGA 1620

GCAAGCCAGG AAGGGAAGGA GTCTGTGTGG TGTGTATGTG CATGCAGCCT ACACCCACAC 1680

GTGTGTACCG GGGGTGAATG TGTGTGAGCA TGTGTGTGTG CATGTACCGG GGAATGAAGG 1740

TGAACATACA CCTCTGTGTG TGCACTGCAG ACACGCCCCA GTGTGTCCAC ATGTGTGTGC 1800

ATGAGTCCAT CTCTGCGCGT GGGGGGGCTC TAACTGCACT TTCGGCCCTT TTGCTCGTGG 1860

GGTCCCACAA GGCCCAGGGC AGTGCCTGCT CCCAGAATCT GGTGCTCTGA CCAGGCCAGG 1920

TGGGGAGGCT TTGGCTGGCT GGGCGTGTAG GACGGTGAGA GCACTTCTGT CTTAAAGGTT 1980

TTTTCTGATT GAAGCTTTAA TGGAGCGTTA TTTATTTATC GAGGCCTCTT TGGTGAGCCT 2040

GGGGAATCAG CAAAAGGGGA GGAGGGGTGT GGGGTTGATA CCCCAACTCC CTCTACCCTT 2100

GAGCAAGGGC AGGGGTCCCT GAGCTGTTCT TCTGCCCCAT ACTGAAGGAA CTGAGGCCTG 2160

GGTGATTTAT TTATTGGGAA AGTGAGGGAG GGAGACAGAC TGACTGACAG CCATGGGTGG 2220

TCAGATGGTG GGGTGGGCCC TCTCCAGGGG GCCAGTTCAG GGCCCAGCTG CCCCCCAGGA 2280

TGGATATGAG ATGGGAGAGG TGAGTGGGGG ACCTTCACTG ATGTGGGCAG GAGGGGTGGT 2340

GAAGGCCTCC CCCAGCCCAG ACCCTGTGGT CCCTCCTGCA GTGTCTGAAG CGCCTGCCTC 2400

CCCACTGCTC TGCCCCACCC TCCAATCTGC ACTTTGATTT GCTTCCTAAC AGCTCTGTTC 2460

CCTCCTGCTT TGGTTTTAAT AAATATTTTG ATGACGTTAA AAAAAGGAAT TCGATAT 2517

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2994 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTCCGGTTTT TCTCAGGGGA CGTTGAAATT ATTTTTGTAA CGGGAGTCGG GAGAGGACGG 60

GGCGTGCCCC GCGTGCGCGC GCGTCGTCCT CCCCGGCGCT CCTCCACAGC TCGCTGGCTC 120

CCGCCGCGGA AAGGCGTCAT GCCGCCCAAA ACCCCCCGAA AAACGGCCGC CACCGCCGCC 180

GCTGCCGCCG CGGAACCCCC GGCACCGCCG CCGCCGCCCC CTCCTGAGGA GGACCCAGAG 240

CAGGACAGCG GCCCGGAGGA CCTGCCTCTC GTCAGGCTTG AGTTTGAAGA AACAGAAGAA 300

CCTGATTTTA CTGCATTATG TCAGAAATTA AAGATACCAG ATCATGTCAG AGAGAGAGCT 360

TGGTTAACTT GGGAGAAAGT TTCATCTGTG GATGGAGTAT TGGGAGGTTA TATTCAAAAG 420

AAAAAGGAAC TGTGGGGAAT CTGTATCTTT ATTGCAGCAG TTGACCTAGA TGAGATGTCG 480

TTCACTTTTA CTGAGCTACA GAAAAACATA GAAATCAGTG TCCATAAATT CTTTAACTTA 540

CTAAAAGAAA TTGATACCAG TACCAAAGTT GATAATGCTA TGTCAAGACT GTTGAAGAAG 600

TATGATGTAT TGTTTGCACT CTTCAGCAAA TTGGAAAGGA CATGTGAACT TATATATTTG 660

ACACAACCCA GCAGTTCGAT ATCTACTGAA ATAAATTCTG CATTGGTGCT AAAAGTTTCT 720

TGGATCACAT TTTTATTAGC TAAAGGGGAA GTATTACAAA TGGAAGATGA TCTGGTGATT 780

TCATTTCAGT TAATGCTATG TGTCCTTGAC TATTTTATTA AACTCTCACC TCCCATGTTG 840

CTCAAAGAAC CATATAAAAC AGCTGTTATA CCCATTAATG GTTCACCTCG AACACCCAGG 900

CGAGGTCAGA ACAGGAGTGC ACGGATAGCA AAACAACTAG AAAATGATAC AAGAATTATT 960

GAAGTTCTCT GTAAAGAACA TGAATGTAAT ATAGATGAGG TGAAAAATGT TTATTTCAAA 1020

AATTTTATAC CTTTTATGAA TTCTCTTGGA CTTGTAACAT CTAATGGACT TCCAGAGGTT 1080

GAAAATCTTT CTAAACGATA CGAAGAAATT TATCTTAAAA ATAAAGATCT AGATGCAAGA 1140

TTATTTTTGG ATCATGATAA AACTCTTCAG ACTGATTCTA TAGACAGTTT TGAAACACAG 1200

AGAACACCAC GAAAAAGTAA CCTTGATGAA GAGGTGAATG TAATTCCTCC ACACACTCCA 1260

GTTAGGACTG TTATGAACAC TATCCAACAA TTAATGATGA TTTTAAATTC AGCAAGTGAT 1320

CAACCTTCAG AAAATCTGAT TTCCTATTTT AACAACTGCA CAGTGAATCC AAAAGAAAGT 1380

ATACTGAAAA GAGTGAAGGA TATAGGATAC ATCTTTAAAG AGAAATTTGC TAAAGCTGTG 1440

GGACAGGGTT GTGTCGAAAT TGGATCACAG CGATACAAAC TTGGAGTTCG CTTGTATTAC 1500

CGAGTAATGG AATCCATGCT TAAATCAGAA GAAGAACGAT TATCCATTCA AAATTTTAGC 1560

AAACTTCTGA ATGACAACAT TTTTCATATG TCTTTATTGG CGTGCGCTCT TGAGGTTGTA 1620

ATGGCCACAT ATAGCAGAAG TACATCTCAG AATCTTGATT CTGGAACAGA TTTGTCTTTC 1680

CCATGGATTC TGAATGTGCT TAATTTAAAA GCCTTTGATT TTTACAAAGT GATCGAAAGT 1740

TTTATCAAAG CAGAAGGCAA CTTGACAAGA GAAATGATAA AACATTTAGA ACGATGTGAA 1800

CATCGAATCA TGGAATCCCT TGCATGGCTC TCAGATTCAC CTTTATTTGA TCTTATTAAA 1860

CAATCAAAGG ACCGAGAAGG ACCAACTGAT CACCTTGAAT CTGCTTGTCC TCTTAATCTT 1920

CCTCTCCAGA ATAATCACAC TGCAGCAGAT ATGTATCTTT CTCCTGTAAG ATCTCCAAAG 1980

AAAAAAGGTT CAACTACGCG TGTAAATTCT ACTGCAAATG CAGAGACACA AGCAACCTCA 2040

GCCTTCCAGA CCCAGAAGCC ATTGAAATCT ACCTCTCTTT CACTGTTTTA TAAAAAAGTG 2100

TATCGGCTAG CCTATCTCCG GCTAAATACA CTTTGTGAAC GCCTTCTGTC TGAGCACCCA 2160

GAATTAGAAC ATATCATCTG GACCCTTTTC CAGCACACCC TGCAGAATGA GTATGAACTC 2220

ATGAGAGACA GGCATTTGGA CCAAATTATG ATGTGTTCCA TGTATGGCAT ATGCAAAGTG 2280

AAGAATATAG ACCTTAAATT CAAAATCATT GTAACAGCAT ACAAGGATCT TCCTCATGCT 2340

GTTCAGGAGA CATTCAAACG TGTTTTGATC AAAGAAGAGG AGTATGATTC TATTATAGTA 2400

TTCTATAACT CGGTCTTCAT GCAGAGACTG AAAACAAATA TTTTGCAGTA TGCTTCCACC 2460

AGGCCCCCTA CCTTGTCACC AATACCTCAC ATTCCTCGAA GCCCTTACAA GTTTCCTAGT 2520

TCACCCTTAC GGATTCCTGG AGGGAACATC TATATTTCAC CCCTGAAGAG TCCATATAAA 2580

ATTTCAGAAG GTCTGCCAAC ACCAACAAAA ATGACTCCAA GATCAAGAAT CTTAGTATCA 2640

ATTGGTGAAT CATTCGGGAC TTCTGAGAAG TTCCAGAAAA TAAATCAGAT GGTATGTAAC 2700

AGCGACCGTG TGCTCAAAAG AAGTGCTGAA GGAAGCAACC CTCCTAAACC ACTGAAAAAA 2760

CTACGCTTTG ATATTGAAGG ATCAGATGAA GCAGATGGAA GTAAACATCT CCCAGGAGAG 2820

TCCAAATTTC AGCAGAAACT GGCAGAAATG ACTTCTACTC GAACACGAAT GCAAAAGCAG 2880

AAAATGAATG ATAGCATGGA TACCTCAAAC AAGGAAGAGA AATGAGGATC TCAGGACCTT 2940

GGTGGACACT GTGTACACCT CTGGATTCAT TGTCTCTCAC AGATGTGACT GTAT 2994

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 928 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Pro Pro Lys Thr Pro Arg Lys Thr Ala Ala Thr Ala Ala Ala Ala

```
1               5                  10                  15
Ala Ala Glu Pro Pro Ala Pro Pro Pro Pro Pro Pro Glu Glu Asp
            20                  25                  30
Pro Glu Gln Asp Ser Gly Pro Glu Asp Leu Pro Leu Val Arg Leu Glu
        35                  40                  45
Phe Glu Glu Thr Glu Glu Pro Asp Phe Thr Ala Leu Cys Gln Lys Leu
    50                  55                  60
Lys Ile Pro Asp His Val Arg Glu Arg Ala Trp Leu Thr Trp Glu Lys
65                  70                  75                  80
Val Ser Ser Val Asp Gly Val Leu Gly Gly Tyr Ile Gln Lys Lys Lys
                85                  90                  95
Glu Leu Trp Gly Ile Cys Ile Phe Ile Ala Ala Val Asp Leu Asp Glu
            100                 105                 110
Met Ser Phe Thr Phe Thr Glu Leu Gln Lys Asn Ile Glu Ile Ser Val
        115                 120                 125
His Lys Phe Phe Asn Leu Leu Lys Glu Ile Asp Thr Ser Thr Lys Val
    130                 135                 140
Asp Asn Ala Met Ser Arg Leu Leu Lys Lys Tyr Asp Val Leu Phe Ala
145                 150                 155                 160
Leu Phe Ser Lys Leu Glu Arg Thr Cys Glu Leu Ile Tyr Leu Thr Gln
                165                 170                 175
Pro Ser Ser Ser Ile Ser Thr Glu Ile Asn Ser Ala Leu Val Leu Lys
            180                 185                 190
Val Ser Trp Ile Thr Phe Leu Leu Ala Lys Gly Glu Val Leu Gln Met
        195                 200                 205
Glu Asp Asp Leu Val Ile Ser Phe Gln Leu Met Leu Cys Val Leu Asp
    210                 215                 220
Tyr Phe Ile Lys Leu Ser Pro Pro Met Leu Leu Lys Glu Pro Tyr Lys
225                 230                 235                 240
Thr Ala Val Ile Pro Ile Asn Gly Ser Pro Arg Thr Pro Arg Arg Gly
                245                 250                 255
Gln Asn Arg Ser Ala Arg Ile Ala Lys Gln Leu Glu Asn Asp Thr Arg
            260                 265                 270
Ile Ile Glu Val Leu Cys Lys Glu His Glu Cys Asn Ile Asp Glu Val
        275                 280                 285
Lys Asn Val Tyr Phe Lys Asn Phe Ile Pro Phe Met Asn Ser Leu Gly
    290                 295                 300
Leu Val Thr Ser Asn Gly Leu Pro Glu Val Glu Asn Leu Ser Lys Arg
305                 310                 315                 320
Tyr Glu Glu Ile Tyr Leu Lys Asn Lys Asp Leu Asp Ala Arg Leu Phe
                325                 330                 335
```

Leu Asp His Asp Lys Thr Leu Gln Thr Asp Ser Ile Asp Ser Phe Glu
340 345 350

Thr Gln Arg Thr Pro Arg Lys Ser Asn Leu Asp Glu Glu Val Asn Val
355 360 365

Ile Pro Pro His Thr Pro Val Arg Thr Val Met Asn Thr Ile Gln Gln
370 375 380

Leu Met Met Ile Leu Asn Ser Ala Ser Asp Gln Pro Ser Glu Asn Leu
385 390 395 400

Ile Ser Tyr Phe Asn Asn Cys Thr Val Asn Pro Lys Glu Ser Ile Leu
405 410 415

Lys Arg Val Lys Asp Ile Gly Tyr Ile Phe Lys Glu Lys Phe Ala Lys
420 425 430

Ala Val Gly Gln Gly Cys Val Glu Ile Gly Ser Gln Arg Tyr Lys Leu
435 440 445

Gly Val Arg Leu Tyr Tyr Arg Val Met Glu Ser Met Leu Lys Ser Glu
450 455 460

Glu Glu Arg Leu Ser Ile Gln Asn Phe Ser Lys Leu Leu Asn Asp Asn
465 470 475 480

Ile Phe His Met Ser Leu Leu Ala Cys Ala Leu Glu Val Val Met Ala
485 490 495

Thr Tyr Ser Arg Ser Thr Ser Gln Asn Leu Asp Ser Gly Thr Asp Leu
500 505 510

Ser Phe Pro Trp Ile Leu Asn Val Leu Asn Leu Lys Ala Phe Asp Phe
515 520 525

Tyr Lys Val Ile Glu Ser Phe Ile Lys Ala Glu Gly Asn Leu Thr Arg
530 535 540

Glu Met Ile Lys His Leu Glu Arg Cys Glu His Arg Ile Met Glu Ser
545 550 555 560

Leu Ala Trp Leu Ser Asp Ser Pro Leu Phe Asp Leu Ile Lys Gln Ser
565 570 575

Lys Asp Arg Glu Gly Pro Thr Asp His Leu Glu Ser Ala Cys Pro Leu
580 585 590

Asn Leu Pro Leu Gln Asn Asn His Thr Ala Ala Asp Met Tyr Leu Ser
595 600 605

Pro Val Arg Ser Pro Lys Lys Lys Gly Ser Thr Thr Arg Val Asn Ser
610 615 620

Thr Ala Asn Ala Glu Thr Gln Ala Thr Ser Ala Phe Gln Thr Gln Lys
625 630 635 640

Pro Leu Lys Ser Thr Ser Leu Ser Leu Phe Tyr Lys Lys Val Tyr Arg
645 650 655

Leu Ala Tyr Leu Arg Leu Asn Thr Leu Cys Glu Arg Leu Leu Ser Glu
660 665 670

```
His Pro Glu Leu Glu His Ile Ile Trp Thr Leu Phe Gln His Thr Leu
        675                 680                 685

Gln Asn Glu Tyr Glu Leu Met Arg Asp Arg His Leu Asp Gln Ile Met
    690                 695                 700

Met Cys Ser Met Tyr Gly Ile Cys Lys Val Lys Asn Ile Asp Leu Lys
705                 710                 715                 720

Phe Lys Ile Ile Val Thr Ala Tyr Lys Asp Leu Pro His Ala Val Gln
                725                 730                 735

Glu Thr Phe Lys Arg Val Leu Ile Lys Glu Glu Glu Tyr Asp Ser Ile
            740                 745                 750

Ile Val Phe Tyr Asn Ser Val Phe Met Gln Arg Leu Lys Thr Asn Ile
        755                 760                 765

Leu Gln Tyr Ala Ser Thr Arg Pro Pro Thr Leu Ser Pro Ile Pro His
    770                 775                 780

Ile Pro Arg Ser Pro Tyr Lys Phe Pro Ser Ser Pro Leu Arg Ile Pro
785                 790                 795                 800

Gly Gly Asn Ile Tyr Ile Ser Pro Leu Lys Ser Pro Tyr Lys Ile Ser
                805                 810                 815

Glu Gly Leu Pro Thr Pro Thr Lys Met Thr Pro Arg Ser Arg Ile Leu
            820                 825                 830

Val Ser Ile Gly Glu Ser Phe Gly Thr Ser Glu Lys Phe Gln Lys Ile
        835                 840                 845

Asn Gln Met Val Cys Asn Ser Asp Arg Val Leu Lys Arg Ser Ala Glu
    850                 855                 860

Gly Ser Asn Pro Pro Lys Pro Leu Lys Lys Leu Arg Phe Asp Ile Glu
865                 870                 875                 880

Gly Ser Asp Glu Ala Asp Gly Ser Lys His Leu Pro Gly Glu Ser Lys
                885                 890                 895

Phe Gln Gln Lys Leu Ala Glu Met Thr Ser Thr Arg Thr Arg Met Gln
            900                 905                 910

Lys Gln Lys Met Asn Asp Ser Met Asp Thr Ser Asn Lys Glu Glu Lys
        915                 920                 925
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3853 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 209..250

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 254..289

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 293..505

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 509..514

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 518..520

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 524..658

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 662..691

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 695..748

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 752..781

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 785..829

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1132..1134

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1138..1149

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 833..862

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG 60

CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG 120

CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC 180

TTAGGGTTAG GCGTTTTGCG CTGCTTCG CGA TGT ACG GGC CAG ATA TAC GCG 232
Arg Cys Thr Gly Gln Ile Tyr Ala
1 5

TTG ACA TTG ATT ATT GAC TAG TTA TTA ATA GTA ATC AAT TAC GGG GTC 280

```
Leu Thr Leu Ile Ile Asp      Leu Leu Ile Val Ile Asn Tyr Gly Val
     10                        1               5

ATT AGT TCA TAG CCC ATA TAT GGA GTT CCG CGT TAC ATA ACT TAC GGT          328
Ile Ser Ser      Pro Ile Tyr Gly Val Pro Arg Tyr Ile Thr Tyr Gly
 10                1                   5                  10

AAA TGG CCC GCC TGG CTG ACC GCC CAA CGA CCC CCG CCC ATT GAC GTC          376
Lys Trp Pro Ala Trp Leu Thr Ala Gln Arg Pro Pro Pro Ile Asp Val
          15                  20                  25

AAT AAT GAC GTA TGT TCC CAT AGT AAC GCC AAT AGG GAC TTT CCA TTG          424
Asn Asn Asp Val Cys Ser His Ser Asn Ala Asn Arg Asp Phe Pro Leu
     30                  35                  40

ACG TCA ATG GGT GGA CTA TTT ACG GTA AAC TGC CCA CTT GGC AGT ACA          472
Thr Ser Met Gly Gly Leu Phe Thr Val Asn Cys Pro Leu Gly Ser Thr
 45                  50                  55                  60

TCA AGT GTA TCA TAT GCC AAG TAC GCC CCC TAT TGA CGT CAA                  514
Ser Ser Val Ser Tyr Ala Lys Tyr Ala Pro Tyr      Arg Gln
                 65                  70              1

TGA CGG TAA ATG GCC CGC CTG GCA TTA TGC CCA GTA CAT GAC CTT ATG          562
    Arg      Met Ala Arg Leu Ala Leu Cys Pro Val His Asp Leu Met
      1        1                   5                  10

GGA CTT TCC TAC TTG GCA GTA CAT CTA CGT ATT AGT CAT CGC TAT TAC          610
Gly Leu Ser Tyr Leu Ala Val His Leu Arg Ile Ser His Arg Tyr Tyr
     15                  20                  25

CAT GGT GAT GCG GTT TTG GCA GTA CAT CAA TGG GCG TGG ATA GCG GTT          658
His Gly Asp Ala Val Leu Ala Val His Gln Trp Ala Trp Ile Ala Val
 30                  35                  40                  45

TGA CTC ACG GGG ATT TCC AAG TCT CCA CCC CAT TGA CGT CAA TGG GAG          706
    Leu Thr Gly Ile Ser Lys Ser Pro Pro His      Arg Gln Trp Glu
      1                   5                  10        1

TTT GTT TTG GCA CCA AAA TCA ACG GGA CTT TCC AAA ATG TCG                  748
Phe Val Leu Ala Pro Lys Ser Thr Gly Leu Ser Lys Met Ser
  5                  10                  15

TAA CAA CTC CGC CCC ATT GAC GCA AAT GGG CGG TAG CGC TGT ACG GTG          796
    Gln Leu Arg Pro Ile Asp Ala Asn Gly Arg      Arg Cys Thr Val
      1                   5                  10        1

GGA GGT CTA TAT AAG CAG AGC TCT CTG GCT AAC TAG AGA ACC CAC TGC          844
Gly Gly Leu Tyr Lys Gln Ser Ser Leu Ala Asn      Arg Thr His Cys
  5                  10                  15        1

TTA CTG GCT TAT CGA AAT TAATACGACT CACTATAGGG AGACCCAAGC                 892
Leu Leu Ala Tyr Arg Asn
  5                  10

TTCGCGCGGG TACCACTCTC TTCCGCATCG CTGTCTGCGA GGGCCAGCTG TTGGGCTCGC       952

GGTTGAGGAC AAACTCTTCG CGGTCTTTCC AGTACTCTTG GATCGGAAAC CCGTCGGCCT      1012

CCGAACGGTA CTCCGCCACC GAGGGACCTG AGCGAGTCCG CATCGACCGG ATCGGAAAAC      1072

CTCTCGAGGC GGCCGCTGCA GTCTAGACGA ATTCGCGTAC GATATCGATG GGCCCTATT       1131
```

```
CTA TAG TGT CAC CTA AAT GCTAGAGCTC GCTGATCAGC CTCGACTGTG              1179
Leu     Cys His Leu Asn
  1       1

CCTTCTAGTT GCCAGCCATC TGTTGTTTGC CCCTCCCCCG TGCCTTCCTT GACCCTGGAA    1239

GGTGCCACTC CCACTGTCCT TTCCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT    1299

AGGTGTCATT CTATTCTGGG GGGTGGGGTG GGGCAGGACA GCAAGGGGGA GGATTGGGAA    1359

GACAATAGCC GAAATGACCG ACCAAGCGAC GCCCAACCTG CCATCACGAG ATTTCGATTC    1419

CACCGCCGCC TTCTATGAAA GGTTGGGCTT CGGAATCGTT TTCCGGGACG CCGGCTGGAT    1479

GATCCTCCAG CGCGGGGATC TCATGCTGGA GTTCTTCGCC CACCCCAACT TGTTTATTGC    1539

AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT    1599

TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGTAT    1659

ACCGTCGACC TCTAGCTAGA GCTTGGCGTA ATCATGGTCA TAGCTGTTTC CTGTGTGAAA    1719

TTGTTATCCG CTCACAATTC CACACAACAT ACGAGCCGGA AGCATAAAGT GTAAAGCCTG    1779

GGGTGCCTAA TGAGTGAGCT AACTCACATT AATTGCGTTG CGCTCACTGC CCGCTTTCCA    1839

GTCGGGAAAC CTGTCGTGCC AGCTGCATTA ATGAATCGGC CAACGCGCGG GGAGAGGCGG    1899

TTTGCGTATT GGGCGCTCTT CCGCTTCCTC GCTCACTGAC TCGCTGCGCT CGGTCGTTCG    1959

GCTGCGGCGA GCGGTATCAG CTCACTCAAA GGCGGTAATA CGGTTATCCA CAGAATCAGG    2019

GGATAACGCA GGAAAGAACA TGTGAGCAAA AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA    2079

GGCCGCGTTG CTGGCGTTTT TCCATAGGCT CCGCCCCCCT GACGAGCATC ACAAAAATCG    2139

ACGCTCAAGT CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC    2199

TGGAAGCTCC CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC    2259

CTTTCTCCCT TCGGGAAGCG TGGCGCTTTC TCAATGCTCA CGCTGTAGGT ATCTCAGTTC    2319

GGTGTAGGTC GTTCGCTCCA AGCTGGGCTG TGTGCACGAA CCCCCCGTTC AGCCCGACCG    2379

CTGCGCCTTA TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC    2439

ACTGGCAGCA GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA    2499

GTTCTTGAAG TGGTGGCCTA ACTACGGCTA CACTAGAAGG ACAGTATTTG GTATCTGCGC    2559

TCTGCTGAAG CCAGTTACCT TCGGAAAAAG AGTTGGTAGC TCTTGATCCG GCAAACAAAC    2619

CACCGCTGGT AGCGGTGGTT TTTTTGTTTG CAAGCAGCAG ATTACGCGCA GAAAAAAAGG    2679

ATCTCAAGAA GATCCTTTGA TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC    2739

ACGTTAAGGG ATTTTGGTCA TGAGATTATC AAAAAGGATC TTCACCTAGA TCCTTTTAAA    2799

TTAAAAATGA AGTTTTAAAT CAATCTAAAG TATATATGAG TAAACTTGGT CTGACAGTTA    2859

CCAATGCTTA ATCAGTGAGG CACCTATCTC AGCGATCTGT CTATTTCGTT CATCCATAGT    2919
```

TGCCTGACTC CCCGTCGTGT AGATAACTAC GATACGGGAG GGCTTACCAT CTGGCCCCAG 2979

TGCTGCAATG ATACCGCGAG ACCCACGCTC ACCGGCTCCA GATTTATCAG CAATAAACCA 3039

GCCAGCCGGA AGGGCCGAGC GCAGAAGTGG TCCTGCAACT TTATCCGCCT CCATCCAGTC 3099

TATTAATTGT TGCCGGGAAG CTAGAGTAAG TAGTTCGCCA GTTAATAGTT TGCGCAACGT 3159

TGTTGCCATT GCTACAGGCA TCGTGGTGTC ACGCTCGTCG TTTGGTATGG CTTCATTCAG 3219

CTCCGGTTCC CAACGATCAA GGCGAGTTAC ATGATCCCCC ATGTTGTGCA AAAAAGCGGT 3279

TAGCTCCTTC GGTCCTCCGA TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT TATCACTCAT 3339

GGTTATGGCA GCACTGCATA ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT 3399

GACTGGTGAG TACTCAACCA AGTCATTCTG AGAATAGTGT ATGCGGCGAC CGAGTTGCTC 3459

TTGCCCGGCG TCAATACGGG ATAATACCGC GCCACATAGC AGAACTTTAA AAGTGCTCAT 3519

CATTGGAAAA CGTTCTTCGG GGCGAAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG 3579

TTCGATGTAA CCCACTCGTG CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT 3639

TTCTGGGTGA GCAAAAACAG GAAGGCAAAA TGCCGCAAAA AAGGGAATAA GGGCGACACG 3699

GAAATGTTGA ATACTCATAC TCTTCCTTTT TCAATATTAT TGAAGCATTT ATCAGGGTTA 3759

TTGTCTCATG AGCGGATACA TATTTGAATG TATTTAGAAA AATAAACAAA TAGGGGTTCC 3819

GCGCACATTT CCCCGAAAAG TGCCACCTGA CGTC 3853

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Arg Cys Thr Gly Gln Ile Tyr Ala Leu Thr Leu Ile Ile Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Leu Leu Ile Val Ile Asn Tyr Gly Val Ile Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Pro Ile Tyr Gly Val Pro Arg Tyr Ile Thr Tyr Gly Lys Trp Pro Ala
 1               5                  10                  15

Trp Leu Thr Ala Gln Arg Pro Pro Pro Ile Asp Val Asn Asn Asp Val
            20                  25                  30

Cys Ser His Ser Asn Ala Asn Arg Asp Phe Pro Leu Thr Ser Met Gly
        35                  40                  45

Gly Leu Phe Thr Val Asn Cys Pro Leu Gly Ser Thr Ser Ser Val Ser
    50                  55                  60

Tyr Ala Lys Tyr Ala Pro Tyr
65                  70
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Arg Gln
 1
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Arg
 1
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Met Ala Arg Leu Ala Leu Cys Pro Val His Asp Leu Met Gly Leu Ser
 1               5                  10                  15

Tyr Leu Ala Val His Leu Arg Ile Ser His Arg Tyr Tyr His Gly Asp
            20                  25                  30

Ala Val Leu Ala Val His Gln Trp Ala Trp Ile Ala Val
        35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Leu Thr Gly Ile Ser Lys Ser Pro Pro His
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Arg Gln Trp Glu Phe Val Leu Ala Pro Lys Ser Thr Gly Leu Ser Lys
 1               5                  10                  15

Met Ser
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Gln Leu Arg Pro Ile Asp Ala Asn Gly Arg
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Arg Cys Thr Val Gly Gly Leu Tyr Lys Gln Ser Ser Leu Ala Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Arg Thr His Cys Leu Leu Ala Tyr Arg Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Leu
1

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Cys His Leu Asn
1

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4026 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 209..250

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 254..289

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 293..505

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 509..514

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 518..520

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 524..658

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 662..691

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 695..748

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 752..781

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 785..829

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 833..862

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1305..1307

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1311..1322

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG      60

CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG     120

CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC     180

TTAGGGTTAG GCGTTTTGCG CTGCTTCG CGA TGT ACG GGC CAG ATA TAC GCG         232
                               Arg Cys Thr Gly Gln Ile Tyr Ala
                                 1               5

TTG ACA TTG ATT ATT GAC TAG TTA TTA ATA GTA ATC AAT TAC GGG GTC       280
Leu Thr Leu Ile Ile Asp     Leu Leu Ile Val Ile Asn Tyr Gly Val
     10                       1                   5

ATT AGT TCA TAG CCC ATA TAT GGA GTT CCG CGT TAC ATA ACT TAC GGT       328
Ile Ser Ser     Pro Ile Tyr Gly Val Pro Arg Tyr Ile Thr Tyr Gly
 10                 1                   5                  10

AAA TGG CCC GCC TGG CTG ACC GCC CAA CGA CCC CCG CCC ATT GAC GTC       376
Lys Trp Pro Ala Trp Leu Thr Ala Gln Arg Pro Pro Pro Ile Asp Val
         15                  20                  25

AAT AAT GAC GTA TGT TCC CAT AGT AAC GCC AAT AGG GAC TTT CCA TTG       424
Asn Asn Asp Val Cys Ser His Ser Asn Ala Asn Arg Asp Phe Pro Leu
     30                  35                  40

ACG TCA ATG GGT GGA CTA TTT ACG GTA AAC TGC CCA CTT GGC AGT ACA       472
Thr Ser Met Gly Gly Leu Phe Thr Val Asn Cys Pro Leu Gly Ser Thr
 45                  50                  55                  60

TCA AGT GTA TCA TAT GCC AAG TAC GCC CCC TAT TGA CGT CAA               514
Ser Ser Val Ser Tyr Ala Lys Tyr Ala Pro Tyr     Arg Gln
                 65                  70          1

TGA CGG TAA ATG GCC CGC CTG GCA TTA TGC CCA GTA CAT GAC CTT ATG       562
    Arg     Met Ala Arg Leu Ala Leu Cys Pro Val His Asp Leu Met
      1       1                   5                  10

GGA CTT TCC TAC TTG GCA GTA CAT CTA CGT ATT AGT CAT CGC TAT TAC       610
Gly Leu Ser Tyr Leu Ala Val His Leu Arg Ile Ser His Arg Tyr Tyr
     15                  20                  25

CAT GGT GAT GCG GTT TTG GCA GTA CAT CAA TGG GCG TGG ATA GCG GTT       658
His Gly Asp Ala Val Leu Ala Val His Gln Trp Ala Trp Ile Ala Val
 30                  35                  40                  45

TGA CTC ACG GGG ATT TCC AAG TCT CCA CCC CAT TGA CGT CAA TGG GAG       706
    Leu Thr Gly Ile Ser Lys Ser Pro Pro His     Arg Gln Trp Glu
      1                   5                  10          1

TTT GTT TTG GCA CCA AAA TCA ACG GGA CTT TCC AAA ATG TCG               748
Phe Val Leu Ala Pro Lys Ser Thr Gly Leu Ser Lys Met Ser
  5                  10                  15

TAA CAA CTC CGC CCC ATT GAC GCA AAT GGG CGG TAG GCG TGT ACG GTG       796
    Gln Leu Arg Pro Ile Asp Ala Asn Gly Arg     Ala Cys Thr Val
      1                   5                  10          1

GGA GGT CTA TAT AAG CAG AGC TCT CTG GCT AAC TAG AGA ACC CAC TGC       844
Gly Gly Leu Tyr Lys Gln Ser Ser Leu Ala Asn     Arg Thr His Cys
  5                  10                  15          1
```

```
TTA CTG GCT TAT CGA AAT TAATACGACT CACTATAGGG AGACCCAAGC                892
Leu Leu Ala Tyr Arg Asn
  5                  10

TTCGCGCGGG TACCACTCTC TTCCGCATCG CTGTCTGCGA GGGCCAGCTG TTGGGCTCGC      952

GGTTGAGGAC AAACTCTTCG CGGTCTTTCC AGTACTCTTG GATCGGAAAC CCGTCGGCCT     1012

CCGAACGGTA CTCCGCCACC GAGGGACCTG AGCGAGTCCG CATCGACCGG ATCGGAAAAC     1072

CTCTCGAGGA ACTGAAAAAC CAGAAAGTTA ACTGGTAAGT TTAGTCTTTT TGTCTTTTTA     1132

TTTCAGGTCC CGGATCCGGT GGTGGTGCAA ATCAAAGAAC TGCTCCTCAG TGGATGTTGC     1192

CTTTACTTCT AGGCCTGTAC GGAAGTGTTA CTTCTGCTCT AAAAGCTGCG GAATTGTACC     1252

CGCGGCCGCT GCAGTCTAGA CGAATTCGCG TACGATATCG ATGGGCCCTA TT CTA         1307
                                                           Leu
                                                            1

TAG TGT CAC CTA AAT GCTAGAGCTC GCTGATCAGC CTCGACTGTG CCTTCTAGTT       1362
    Cys His Leu Asn
      1

GCCAGCCATC TGTTGTTTGC CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC     1422

CCACTGTCCT TTCCTAATAA AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT     1482

CTATTCTGGG GGGTGGGGTG GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCC     1542

GAAATGACCG ACCAAGCGAC GCCCAACCTG CCATCACGAG ATTTCGATTC CACCGCCGCC     1602

TTCTATGAAA GGTTGGGCTT CGGAATCGTT TTCCGGGACG CCGGCTGGAT GATCCTCCAG     1662

CGCGGGGATC TCATGCTGGA GTTCTTCGCC CACCCCAACT TGTTTATTGC AGCTTATAAT     1722

GGTTACAAAT AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT     1782

TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGTAT ACCGTCGACC     1842

TCTAGCTAGA GCTTGGCGTA ATCATGGTCA TAGCTGTTTC CTGTGTGAAA TTGTTATCCG     1902

CTCACAATTC CACACAACAT ACGAGCCGGA AGCATAAAGT GTAAAGCCTG GGGTGCCTAA     1962

TGAGTGAGCT AACTCACATT AATTGCGTTG CGCTCACTGC CCGCTTTCCA GTCGGGAAAC     2022

CTGTCGTGCC AGCTGCATTA ATGAATCGGC CAACGCGCGG GGAGAGGCGG TTTGCGTATT     2082

GGGCGCTCTT CCGCTTCCTC GCTCACTGAC TCGCTGCGCT CGGTCGTTCG GCTGCGGCGA     2142

GCGGTATCAG CTCACTCAAA GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA     2202

GGAAAGAACA TGTGAGCAAA AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG     2262

CTGGCGTTTT TCCATAGGCT CCGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT     2322

CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAGCTCC     2382

CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT     2442

TCGGGAAGCG TGGCGCTTTC TCAATGCTCA CGCTGTAGGT ATCTGAGTTC GGTGTAGGTC     2502
```

GTTCGCTCCA AGCTGGGCTG TGTGCACGAA CCCCCCGTTC AGCCCGACCG CTGCGCCTTA 2562

TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA 2622

GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG 2682

TGGTGGCCTA ACTACGGCTA CACTAGAAGG ACAGTATTTG GTATCTGCGC TCTGCTGAAG 2742

CCAGTTACCT TCGGAAAAAG AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT 2802

AGCGGTGGTT TTTTTGTTTG CAAGCAGCAG ATTACGCGCA GAAAAAAAGG ATCTCAAGAA 2862

GATCCTTTGA TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG 2922

ATTTTGGTCA TGAGATTATC AAAAAGGATC TTCACCTAGA TCCTTTTAAA TTAAAAATGA 2982

AGTTTTAAAT CAATCTAAAG TATATATGAG TAAACTTGGT CTGACAGTTA CCAATGCTTA 3042

ATCAGTGAGG CACCTATCTC AGCGATCTGT CTATTTCGTT CATCCATAGT TGCCTGACTC 3102

CCCGTCGTGT AGATAACTAC GATACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG 3162

ATACCGCGAG ACCCACGCTC ACCGGCTCCA GATTTATCAG CAATAAACCA GCCAGCCGGA 3222

AGGGCCGAGC GCAGAAGTGG TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT 3282

TGCCGGGAAG CTAGAGTAAG TAGTTCGCCA GTTAATAGTT TGCGCAACGT TGTTGCCATT 3342

GCTACAGGCA TCGTGGTGTC ACGCTCGTCG TTTGGTATGG CTTCATTCAG CTCCGGTTCC 3402

CAACGATCAA GGCGAGTTAC ATGATCCCCC ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC 3462

GGTCCTCCGA TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT TATCACTCAT GGTTATGGCA 3522

GCACTGCATA ATTCTCTTAC TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG 3582

TACTCAACCA AGTCATTCTG AGAATAGTGT ATGCGGCGAC CGAGTTGCTC TTGCCCGGCG 3642

TCAATACGGG ATAATACCGC GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA 3702

CGTTCTTCGG GGCGAAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA 3762

CCCACTCGTG CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA 3822

GCAAAAACAG GAAGGCAAAA TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA 3882

ATACTCATAC TCTTCCTTTT TCAATATTAT TGAAGCATTT ATCAGGGTTA TTGTCTCATG 3942

AGCGGATACA TATTTGAATG TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATTT 4002

CCCCGAAAAG TGCCACCTGA CGTC 4026

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Arg Cys Thr Gly Gln Ile Tyr Ala Leu Thr Leu Ile Ile Asp
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Leu Leu Ile Val Ile Asn Tyr Gly Val Ile Ser Ser
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Pro Ile Tyr Gly Val Pro Arg Tyr Ile Thr Tyr Gly Lys Trp Pro Ala
 1               5                  10                  15

Trp Leu Thr Ala Gln Arg Pro Pro Pro Ile Asp Val Asn Asn Asp Val
            20                  25                  30

Cys Ser His Ser Asn Ala Asn Arg Asp Phe Pro Leu Thr Ser Met Gly
        35                  40                  45

Gly Leu Phe Thr Val Asn Cys Pro Leu Gly Ser Thr Ser Ser Val Ser
    50                  55                  60

Tyr Ala Lys Tyr Ala Pro Tyr
65                  70
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Arg Gln
 1
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Arg
1

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met Ala Arg Leu Ala Leu Cys Pro Val His Asp Leu Met Gly Leu Ser
1 5 10 15

Tyr Leu Ala Val His Leu Arg Ile Ser His Arg Tyr Tyr His Gly Asp
20 25 30

Ala Val Leu Ala Val His Gln Trp Ala Trp Ile Ala Val
35 40 45

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Leu Thr Gly Ile Ser Lys Ser Pro Pro His
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Arg Gln Trp Glu Phe Val Leu Ala Pro Lys Ser Thr Gly Leu Ser Lys
1 5 10 15

Met Ser

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gln Leu Arg Pro Ile Asp Ala Asn Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ala Cys Thr Val Gly Gly Leu Tyr Lys Gln Ser Ser Leu Ala Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Arg Thr His Cys Leu Leu Ala Tyr Arg Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Leu
1

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Cys His Leu Asn
1

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4249 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 209..250

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 254..289

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 293..505

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 509..514

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 518..520

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 524..658

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 662..691

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 695..748

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 752..781

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 785..829

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 833..862

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1528..1530

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1534..1545

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
GACGGATCGG GAGATCTCCC GATCCCCTAT GGTCGACTCT CAGTACAATC TGCTCTGATG      60

CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG     120

CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC     180

TTAGGGTTAG GCGTTTTGCG CTGCTTCG CGA TGT ACG GGC CAG ATA TAC GCG         232
                               Arg Cys Thr Gly Gln Ile Tyr Ala
                                 1               5

TTG ACA TTG ATT ATT GAC TAG TTA TTA ATA GTA ATC AAT TAC GGG GTC         280
Leu Thr Leu Ile Ile Asp     Leu Leu Ile Val Ile Asn Tyr Gly Val
     10                       1                   5

ATT AGT TCA TAG CCC ATA TAT GGA GTT CCG CGT TAC ATA ACT TAC GGT         328
Ile Ser Ser     Pro Ile Tyr Gly Val Pro Arg Tyr Ile Thr Tyr Gly
 10                 1                   5                  10

AAA TGG CCC GCC TGG CTG ACC GCC CAA CGA CCC CCG CCC ATT GAC GTC         376
Lys Trp Pro Ala Trp Leu Thr Ala Gln Arg Pro Pro Pro Ile Asp Val
         15                  20                  25

AAT AAT GAC GTA TGT TCC CAT AGT AAC GCC AAT AGG GAC TTT CCA TTG         424
Asn Asn Asp Val Cys Ser His Ser Asn Ala Asn Arg Asp Phe Pro Leu
     30                  35                  40

ACG TCA ATG GGT GGA CTA TTT ACG GTA AAC TGC CCA CTT GGC AGT ACA         472
Thr Ser Met Gly Gly Leu Phe Thr Val Asn Cys Pro Leu Gly Ser Thr
 45                  50                  55                  60

TCA AGT GTA TCA TAT GCC AAG TAC GCC CCC TAT TGA CGT CAA                 514
Ser Ser Val Ser Tyr Ala Lys Tyr Ala Pro Tyr     Arg Gln
                 65                  70          1

TGA CGG TAA ATG GCC CGC CTG GCA TTA TGC CCA GTA CAT GAC CTT ATG         562
    Arg     Met Ala Arg Leu Ala Leu Cys Pro Val His Asp Leu Met
      1       1                   5                  10

GGA CTT TCC TAC TTG GCA GTA CAT CTA CGT ATT AGT CAT CGC TAT TAC         610
Gly Leu Ser Tyr Leu Ala Val His Leu Arg Ile Ser His Arg Tyr Tyr
```

```
     15                  20                  25

CAT GGT GAT GCG GTT TTG GCA GTA CAT CAA TGG GCG TGG ATA GCG GTT        658
His Gly Asp Ala Val Leu Ala Val His Gln Trp Ala Trp Ile Ala Val
 30                  35                  40                  45

TGA CTC ACG GGG ATT TCC AAG TCT CCA CCC CAT TGA CGT CAA TGG GAG        706
    Leu Thr Gly Ile Ser Lys Ser Pro Pro His     Arg Gln Trp Glu
      1                 5                  10       1

TTT GTT TTG GCA CCA AAA TCA ACG GGA CTT TCC AAA ATG TCG                748
Phe Val Leu Ala Pro Lys Ser Thr Gly Leu Ser Lys Met Ser
  5                  10                  15

TAA CAA CTC CGC CCC ATT GAC GCA AAT GGG CGG TAG GCG TGT ACG GTG        796
    Gln Leu Arg Pro Ile Asp Ala Asn Gly Arg     Ala Cys Thr Val
      1                 5                  10       1

GGA GGT CTA TAT AAG CAG AGC TCT CTG GCT AAC TAG AGA ACC CAC TGC        844
Gly Gly Leu Tyr Lys Gln Ser Ser Leu Ala Asn     Arg Thr His Cys
  5                  10                  15       1

TTA CTG GCT TAT CGA AAT TAATACGACT CACTATAGGG AGACCCAAGC               892
Leu Leu Ala Tyr Arg Asn
  5                  10

TTCGCGCGGG TACCACTCTC TTCCGCATCG CTGTCTGCGA GGGCCAGCTG TTGGGCTCGC      952

GGTTGAGGAC AAACTCTTCG CGGTCTTTCC AGTACTCTTG GATCGGAAAC CCGTCGGCCT     1012

CCGAACGGTA CTCCGCCACC GAGGGACCTG AGCGAGTCCG CATCGACCGG ATCGGAAAAC     1072

CTCTCGAGGA ACTGAAAAAC CAGAAAGTTA ACTGGTAAGT TTAGTCTTTT TGTCTTTTTA     1132

TTTCAGGTCC CGGATCTGAG TTAGGGCGGG ACATGGGCGG AGTTAGGGGC GGGACTATGG     1192

TTGCTGACTA ATTGAGATGC ATGCTTTGCA TACTTCTGCC TGCTGGGGAG CCTGGGGACT     1252

TTCCACACCT GGTTGCTGAC TAATTGAGAT GCATGCTTTG CATACTTCTG CCTGCTGGGG     1312

AGCCTGGGGA CTTTCCACAC CCTAACTGAC ACACATTCCA CAGCTGGTTC TTTCAGATCC     1372

GGTGGTGGTG CAAATCAAAG AACTGCTCCT CAGTGGATGT TGCCTTTACT TCTAGGCCTG     1432

TACGGAAGTG TTACTTCTGC TCTAAAAGCT GCGGAATTGT ACCCGCGGCC GCTGCAGTCT     1492

AGACGAATTC GCGTACGATA TCGATGGGCC CTATT CTA TAG TGT CAC CTA AAT         1545
                                      Leu     Cys His Leu Asn
                                        1       1

GCTAGAGCTC GCTGATCAGC CTCGACTGTG CCTTCTAGTT GCCAGCCATC TGTTGTTTGC     1605

CCCTCCCCCG TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA     1665

AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG GGGTGGGGTG     1725

GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCC GAAATGACCG ACCAAGCGAC     1785

GCCCAACCTG CCATCACGAG ATTTCGATTC CACCGCCGCC TTCTATGAAA GGTTGGGCTT     1845

CGGAATCGTT TTCCGGGACG CCGGCTGGAT GATCCTCCAG CGCGGGGATC TCATGCTGGA     1905
```

GTTCTTCGCC CACCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG 1965

CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA 2025

ACTCATCAAT GTATCTTATC ATGTCTGTAT ACCGTCGACC TCTAGCTAGA GCTTGGCGTA 2085

ATCATGGTCA TAGCTGTTTC CTGTGTGAAA TTGTTATCCG CTCACAATTC CACACAACAT 2145

ACGAGCCGGA AGCATAAAGT GTAAAGCCTG GGGTGCCTAA TGAGTGAGCT AACTCACATT 2205

AATTGCGTTG CGCTCACTGC CCGCTTTCCA GTCGGGAAAC CTGTCGTGCC AGCTGCATTA 2265

ATGAATCGGC CAACGCGCGG GGAGAGGCGG TTTGCGTATT GGGCGCTCTT CCGCTTCCTC 2325

GCTCACTGAC TCGCTGCGCT CGGTCGTTCG GCTGCGGCGA GCGGTATCAG CTCACTCAAA 2385

GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA GGAAAGAACA TGTGAGCAAA 2445

AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT TCCATAGGCT 2505

CCGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT CAGAGGTGGC GAAACCCGAC 2565

AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAGCTCC CTCGTGCGCT CTCCTGTTCC 2625

GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT TCGGGAAGCG TGGCGCTTTC 2685

TCAATGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC GTTCGCTCCA AGCTGGGCTG 2745

TGTGCACGAA CCCCCCGTTC AGCCCGACCG CTGCGCCTTA TCCGGTAACT ATCGTCTTGA 2805

GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA GCCACTGGTA ACAGGATTAG 2865

CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG TGGTGGCCTA ACTACGGCTA 2925

CACTAGAAGG ACAGTATTTG GTATCTGCGC TCTGCTGAAG CCAGTTACCT TCGGAAAAAG 2985

AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGTGGTT TTTTTGTTTG 3045

CAAGCAGCAG ATTACGCGCA GAAAAAAAGG ATCTCAAGAA GATCCTTTGA TCTTTTCTAC 3105

GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG ATTTTGGTCA TGAGATTATC 3165

AAAAAGGATC TTCACCTAGA TCCTTTTAAA TTAAAAATGA AGTTTTAAAT CAATCTAAAG 3225

TATATATGAG TAAACTTGGT CTGACAGTTA CCAATGCTTA ATCAGTGAGG CACCTATCTC 3285

AGCGATCTGT CTATTTCGTT CATCCATAGT TGCCTGACTC CCCGTCGTGT AGATAACTAC 3345

GATACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG ATACCGCGAG ACCCACGCTC 3405

ACCGGCTCCA GATTTATCAG CAATAAACCA GCCAGCCGGA AGGGCCGAGC GCAGAAGTGG 3465

TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT TGCCGGGAAG CTAGAGTAAG 3525

TAGTTCGCCA GTTAATAGTT TGCGCAACGT TGTTGCCATT GCTACAGGCA TCGTGGTGTC 3585

ACGCTCGTCG TTTGGTATGG CTTCATTCAG CTCCGGTTCC CAACGATCAA GGCGAGTTAC 3645

ATGATCCCCC ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC GGTCCTCCGA TCGTTGTCAG 3705

AAGTAAGTTG GCCGCAGTGT TATCACTCAT GGTTATGGCA GCACTGCATA ATTCTCTTAC 3765

TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG TACTCAACCA AGTCATTCTG 3825

AGAATAGTGT ATGCGGCGAC CGAGTTGCTC TTGCCCGGCG TCAATACGGG ATAATACCGC 3885

GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA CGTTCTTCGG GGCGAAAACT 3945

CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA CCCACTCGTG CACCCAACTG 4005

ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG GAAGGCAAAA 4065

TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA ATACTCATAC TCTTCCTTTT 4125

TCAATATTAT TGAAGCATTT ATCAGGGTTA TTGTCTCATG AGCGGATACA TATTTGAATG 4185

TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATTT CCCCGAAAAG TGCCACCTGA 4245

CGTC 4249

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Arg Cys Thr Gly Gln Ile Tyr Ala Leu Thr Leu Ile Ile Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Leu Leu Ile Val Ile Asn Tyr Gly Val Ile Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Pro Ile Tyr Gly Val Pro Arg Tyr Ile Thr Tyr Gly Lys Trp Pro Ala
1 5 10 15

Trp Leu Thr Ala Gln Arg Pro Pro Pro Ile Asp Val Asn Asn Asp Val
20 25 30

Cys Ser His Ser Asn Ala Asn Arg Asp Phe Pro Leu Thr Ser Met Gly
35 40 45

Gly Leu Phe Thr Val Asn Cys Pro Leu Gly Ser Thr Ser Ser Val Ser
50 55 60

Tyr Ala Lys Tyr Ala Pro Tyr
65 70

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Arg Gln
1

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Arg
1

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Ala Arg Leu Ala Leu Cys Pro Val His Asp Leu Met Gly Leu Ser
1 5 10 15

Tyr Leu Ala Val His Leu Arg Ile Ser His Arg Tyr Tyr His Gly Asp
20 25 30

Ala Val Leu Ala Val His Gln Trp Ala Trp Ile Ala Val
35 40 45

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Leu Thr Gly Ile Ser Lys Ser Pro Pro His
1 5 10

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Arg Gln Trp Glu Phe Val Leu Ala Pro Lys Ser Thr Gly Leu Ser Lys
1 5 10 15

Met Ser

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Gln Leu Arg Pro Ile Asp Ala Asn Gly Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Ala Cys Thr Val Gly Gly Leu Tyr Lys Gln Ser Ser Leu Ala Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Arg Thr His Cys Leu Leu Ala Tyr Arg Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Leu
1

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Cys His Leu Asn
1